THE INFLUENCE OF EARLY LIFE SOCIAL ENVIRONMENTS ON THE DEVELOPMENT OF NONAPEPTIDE RECEPTORS AND SOCIAL BEHAVIOR IN THE PRAIRIE VOLE

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ABSTRACT

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Social environments shape behavior and neurobiology across the lifetime. This phenotypic shaping is a dynamic process that involves sequences of distinct social environments across stages of development. Unfortunately, research on the environmental effects on brain development and behavior too often focuses on individual periods of life. This prevents an understanding of how sequences of life stages could interactively shape phenotypes over developmental time. Here I first describe the development of oxytocin and vasopressin receptors in the prairie vole (*Microtus ochrogaster*) and demonstrate the influence of juvenile social and spatial enrichment on brain development. These developmental trajectories and their responses to enrichment are highly region-specific, and somewhat sex-specific. Notably, the general patterns of trajectories for oxytocin receptors (OTR) and for vasopressin receptors (V1aR) are highly distinct. Enrichment results in a male-specific reduction of V1aR across several brain regions, whereas OTR is strictly promoted following enrichment. I then demonstrate the interactive impacts of perinatal and juvenile social environments on oxytocin and vasopressin receptor development and social cognition. Male prairie voles that experience a two-hit model of social deprivation (single-mother rearing + social isolation) develop higher oxytocin and vasopressin receptors in specific regions, and also develop impaired social discrimination. Lastly, I provide support for how perinatal and juvenile social environments can interact to influence the behavioral effects of intranasal oxytocin. Males experiencing a two-hit model of social deprivation (single-mother rearing + social isolation) engage in promoted alloparental care

behavior towards novel neonatal voles. Taken together, this research highlights the influence of interactive effects of consecutive social environments on neurobiology and behavior, and emphasizes the importance of considering multi-factorial dynamics on the developmental process in future studies.

BIOGRAPHICAL SKETCH

Early in his academic career, George was broadly interested in cross-species and cross-population differences in behavior. During a summer research fellowship as an undergraduate student at SUNY College of Environmental Science & Forestry, George devised a project to explore the comparative space use of woodland and meadow jumping mice in deciduous forests in the Adirondacks of New York. He was particularly drawn to the perception of risk in natural environments and wanted to study the effects of habitat edges (such as the transition zone between a forest and a meadow) on differential perceived risk in these two species. In the same summer that he would develop an appreciation for the difficulty of trapping a sufficient number of jumping mice for meaningful analysis, George devised a second project that examined risk-perception in a far more off-kilter way: he studied the effects of the presence of a mouse cadaver on perceived risk in wild small mammal populations, using avoidance of experimental food trays as a proxy for perceived risk. Compared to control food trays containing novel objects or only seeds, George found that experimental food trays containing a mouse cadaver had significantly more seeds remaining intact every night. George followed up his studies of necrophobic behavior in the lab under the mentorship of Dr. William Shields and demonstrated a unique avoidance of mouse cadavers by *Mus musculus* in a Y-maze. Compared to interactions with a novel object in the Y-maze, mice would engage in a unique avoidance behavior towards the cadaver, avoiding contact when passing the cadaver in the maze arm, and exploring regions of the arm beyond the cadaver significantly less than during trials with a novel object. This research has been published in *Behavioural Processes*. As a recipient of an NSF-REU fellowship with Humboldt State University, George conducted research on the effects of cattle-grazing on space use of *Peromyscus* mice in meadows throughout the Sierra Nevada mountains of California. He found that the habitat-altering effects of cattle-grazing on vegetation heights resulted in increased space

use behavior. Overall, these experiences shaped a deep interest in the factors that affect risk-related space use in natural contexts.

In his final year at SUNY-ESF, George became familiar with the research of Dr. Alexander Ophir and became fascinated by the idea of studying the neurobiologial mechanisms that mediate the array of social behaviors across rodent taxa. George immediately enrolled in a graduate level course entitled "Advanced Neuroscience" at SUNY Upstate Medical University during his final semester at SUNY-ESF. This class featured a weekly rotation of lecturers with different expertise across cutting-edge subfields of neuroscience. With relentless, and impassioned daily effort to digest this radically unfamiliar course material, George finished with the top grade as the only undergraduate student in the class of 20 M.D. and Ph.D. students, and certainly the only student with a predominant background in ecology. This was a major turning-point for George, as he realized his passion and potential for studying the neural basis of behavior. George was in frequent contact with Dr. Ophir during this time, who would go on to accept him into his lab. Under the mentorship of Dr. Alexander Ophir at Cornell University, George discovered his particular interest in questions about the neural basis of ontogenetic changes in social behavior.

DEDICATION

To Sarah Jane, the single mother who raised me in an enriching world of small critters

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The social environment of Uris Hall was one for stress buffering, and shaped me into a more complete developmental neuroethologist. I have many people to thank for this, and will do my best to acknowledge the main players here. First and foremost, I must acknowledge my committee members.

I was blessed to cross paths with Dr. Michael Goldstein, one of the most infectious enthusiasts for developmental neurobiology and psychology, at this early stage in my academic career. I was constantly inspired by Mike's genuine passion for teaching and discussing developmental research. One of my biggest regrets is that I did not take every single developmental seminar that he offered. So many of my thoughts on developmental neurobiology came to me after conversations in his seminars, or following his reading suggestions.

With Dr. David Smith, I deeply explored my passion for systems behavioral neuroscience (and Pacers basketball). The seminars and conversations I shared with David will continue to inspire my pursuits to describe the neural basis of social behavior with fine scale precision. More than that, I can count dozens of times when David stopped me in the hall to have de-stressing conversations about our shared passion for basketball. These go a long way towards creating a comforting environment in the workplace.

I am privileged to have been able to develop an academic relationship with Dr. Elizabeth Adkins-Regan. Teaching the discussion sections in Elizabeth's Hormones and Behavior class was one of the greatest honors during my time at Cornell. As a committee member, Elizabeth helped me appreciate the importance of understanding interactions between neuroendocrine systems. It meant a great deal to me to receive advisement and praise from one of the most prominent figures in comparative neuroendocrinology. At times when I waiver in self-

confidence, I will continue to recall the praise that Elizabeth would give me after talks and after my qualifying exam.

I could write a 100 page acknowledgement for my advisor, Dr. Alexander Ophir, and still not feel I was doing him justice. Alex opened up more doors than I can count, and saw me through the most challenging time in my life. Alex's belief in me is something I cherish beyond words. It has helped me withstand many obstacles, and is a critical source of my self confidence to make a career in research and professorship. I find myself playing Alex's words of wisdom through my head on a weekly basis.

I also must thank my fellow graduate students, in particular my lab mate Marissa Rice who was office mate and close friend during 6 years of study between Oklahoma State University and Cornell. I also must thank Erin Isbilen and Samantha Carouso for being so persistent in getting me out of my social isolation when I really needed it these last two years.

I owe thanks to Ryan Kalinowski, Dr. Bill Shields, Dr. Martin Schlaepfer, and Dr. Jacqueline Frair for giving me my first independent research opportunities.

I wouldn't have been able to make it to this point without the love of my family. Especially crucial to my completion of this dissertation was the support of my partner, Cindy Cordoba. Cindy provided a constant flow of love, nourishment, and reprieve over these last few months when I needed it the most. I am truly blessed to be with someone who is so supportive of my intellectual and professional pursuits.

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CHAPTER 1 – General Introduction

Social environments dynamically shape the neurobiology and behavior of the developing organism. They establish the social interactions through which we acquire resources and learn about the world. As an organism develops, they transition between distinct social environments and the interactions they afford. For example, the mammalian embryo experiences a relatively limited social environment, with practically all resources and information being filtered through the mother. At birth, the social environment becomes closely defined by interactions with parents and kin. A mother rat cues the stressors of the environment to the neonate through the quality of care it provides. A female neonatal rat that receives low amounts of licking and grooming will be more likely to develop a high anxiety phenotype and they too will provide low levels of care to their offspring (Champagne & Meaney, 2007; D. Francis, Diorio, Liu, & Meaney, 1999). Adolescence marks a transition into a social environment largely defined by interactions with non-kin. Despite the relative reduction of neuroplasticity at this point in development, variable social experiences during adolescence (e.g., isolation versus enrichment, social defeat versus play fighting) result in long-lasting changes to neurobiology and behavior (Burke, McCormick, Pellis, & Lukkes, 2017).

By the time an organism has progressed through these distinct social environments and reaches full adulthood, their neurobiology and behavior has been dynamically shaped by the various social experiences along the way. Unfortunately, a glaring majority of the research on developmental neurobiology and behavior focuses on the environmental effects of single stages of life in isolation from those occurring before or after. This approach prevents us from understanding how the neurobiology and behavior of an organism can be dynamically shaped by

the sequence of social environments they experience. It is particularly important to understand the potential for compounding effects of consecutive social environments on the development of social behavior. For example, an early social environment that shapes an individual towards an anti-social phenotype will influence the way that individual engages with social environments later in life, thereby affecting the degree to which these later social environments will shape the individual. It is crucial, therefore, to identify how sequences of early life social environments dynamically shape the neural mechanisms that support social behavior.

The nonapeptides oxytocin (OT) and vasopressin (VP), and their non-mammalian homologs, have been identified as key players in the mechanisms of social behavior across vertebrate species (Goodson, 2005; Johnson & Young, 2017). As neuromodulators, OT and VP can influence neural circuits that mediate a variety of social behaviors, including social attachment, parental care, social recognition and aggression (Albers, 2012; Lee, Macbeth, Pagani, & Young, 2009). In mammals, OT and VP are released from neurons in the hypothalamus, specifically the paraventricular nucleus and suprachiasmic nucleus. A variety of social experiences can induce these neurons to release OT and VP. For example, OT is released during touch (Okabe, Yoshida, Takayanagi, & Onaka, 2015) and suckling in rats (Veenema & Neumann, 2008). In this way OT and VP can mediate the effects of the social environment on neural development and behavior (Bales & Perkeybile, 2012).

Receptors for OT and VP are key features of nonapeptide systems, dictating binding across different regions of the brain depending on the patterns and densities of their expression. By this mechanism, variation of oxytocin receptor (OTR) and vasopressin 1a receptor (V1aR) densities

supports species-differences and sex-differences in social behavior (Albers, 2015; Beery, Lacey, & Francis, 2008; Dumais & Veenema, 2016). Social environments can impact the expression of these receptors and provide a mechanism for variable behavioral phenotypes later in life (Bales & Perkeybile, 2012). For example, rat pups that receive low levels of maternal care develop lower densities of OTR in the specific regions of the brain, and this is linked to the antisocial effects of this rearing environment on behavior that was discussed earlier (D. D. Francis, Champagne, & Meaney, 2000).

The prairie vole (*Microtus ochrogaster*) has provided a bounty of information about the role of OT and VP systems in parental care and attachment behavior (Young, Gobrogge, Liu, & Wang, 2011). This is largely due to three rare features of prairie vole ethology: the expression of alloparental care in pre-adolescents, the formation of pair bonds between adults, and the bi-parental care of offspring (Carter, Devries, & Getz, 1995; Getz, Carter, & Gavish, 1981; Kenkel, Perkeybile, & Carter, 2017). Of equal value to their study is the amount of individual variation in the degree to which these behaviors are expressed (Ophir, Gessel, Zheng, & Phelps, 2012; Vogel, Patisaul, Arambula, Tiezzi, & McGraw, 2018). Identifying the source of this variation in behavior is a major goal of researchers that study the role of social environments on OT and VP system development in the prairie vole. For example, prairie voles that are reared by a single-mother (compared to those reared by a bi-parental unit) develop lower alloparental care behavior (females only) and impaired partner preference behavior (Ahern & Young, 2009). These single-mother reared females also develop higher expressions of OT mRNA in the paraventricular nucleus (Ahern & Young, 2009). Social environments experienced later in life can also impact OT systems and behavior. Male prairie voles that experience social isolation during juvenile and

adolescent life stages develop high anxiety behavior and enhanced mRNA expression for OT and VP in the paraventricular nucleus (Pan, Liu, Young, Zhang, & Wang, 2009). Social isolation in adulthood results in similar effects to mRNA expression in female prairie voles, and the development of depressive-like behavior in both males and females (Grippo, Cushing, & Carter, 2007). Research of this kind has been limited to a focus on the effects of social environments occurring at single stages of life. For my dissertation, I investigated the independent and combined effects of perinatal (i.e., 'pre-weaning') and pre-adult (i.e., juvenile and adolescent, or 'post-weaning') social environments on the development of nonapeptide systems and social behavior of the prairie vole.

My first research aim was to thoroughly describe changes in OTR and V1aR expression across development in prairie voles (Chapter 2). In the initial phase of this project, I explored the ontogeny of OTR and V1aR across twenty-six brain regions in both males and females. I identified region-specific developmental trajectories of OTR and V1aR expression, with receptor densities either increasing with age, decreasing with age, peaking during late pre-weaning, or remaining constant over time depending on the region. This project provides the most thorough account of prairie vole OTR and V1aR ontogeny to date, being the first to describe most of the regions with known expression, and the first to do so in both sexes. These patterns of OTR and V1aR ontogeny in prairie voles can now be compared to similarly thorough developmental descriptions of these receptors in rats and mice (Hammock & Levitt, 2013; Smith et al., 2017). This reveals compelling species differences and mechanisms by which species-specific patterns of OTR and V1aR ontogeny may support species-specific development of social behavior.

A second phase of this project explored the impacts of post-weaning social and spatial enrichment on these identified trajectories of OTR and V1aR (Chapter 2). In both males and females, OTR expression was promoted across select regions following enrichment. However, in males only, V1aR was reduced in several regions following enrichment. This marked the first report on the effects of environmental enrichment on OTR and V1aR expression in the prairie vole. Further, this project established a foundation for exploring the effects social environments on OTR and V1aR trajectories across stages of development.

As previously discussed, being reared by a single mother or by a bi-parental unit has long term consequences on OT systems and social behavior (Ahern & Young, 2009). Similarly, experiencing social isolation or group housing during adolescence and adulthood can further shape these phenotypes (Grippo et al., 2007; Pan et al., 2009). The second research aim of my dissertation was to explore whether pre-weaning and post-weaning social environments can interactively shape OTR, V1aR and social cognition (Chapter 3). I found that males reared by a single-mother expressed higher V1aR in two regions (the retrosplenial cortex and medial amygdala). In addition, males that experienced post-weaning social isolation had higher OTR in a few regions (the prefrontal cortex, septohippocampal nucleus, and basolateral amygdala). It was only males that experienced both single mother rearing and post-weaning social isolation that exhibited higher OTR in the lateral septum and that also demonstrated impaired social demonstration. This marked the first demonstration that relative social deprivation at two distinct periods of development can have combinatorial effects on neural systems and behavior of the prairie vole.

Manipulating OT levels through exogenous application can result in long-term changes to OT systems and social behavior (Bales et al., 2007). For example, continuous infusion of OT into the brain results in significant reduction of OTR throughout (Insel, Winslow, & Witt, 1992). Intranasal oxytocin has recently emerged as a noninvasive means of manipulating OT levels peripherally and centrally in rodent species (Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013). Male prairie voles that receive chronic intranasal treatments develop impairments to pair bonding behavior as adults (Bales et al., 2013). For my third research aim, I explored whether social environments would mediate the response of male prairie voles to intranasal oxytocin (Chapter 4). I replicated the two rearing conditions that produced the most distinct OTR phenotype in my previous project. Male voles that were reared by a single mother and then housed in social isolation engaged in more social contact with novel juvenile voles, and were more likely to form a partner preference, regardless of intranasal treatment (saline or oxytocin). This effect on behavior makes it plausible that our early life manipulation affected OT phenotypes. To this point, males reared by a single mother that were then housed in isolation responded to chronic dosing with intranasal oxytocin in a distinct way. These males engaged in the highest amounts of alloparental care, seemingly linked to an inhibition of attack behavior towards the novel pups. To my knowledge, this study marks the first report of manipulated social environments affecting the behavioral response to intranasal oxytocin.

In the concluding section, I synthesize major findings and address common themes across the three research chapters. I also discuss the importance of determining how environmental alterations of OT and VP systems may affect social behavior via interactions with other neurobiological systems. In closing, I propose future directions to address adaptive features of

phenotypic shaping of OT and VP systems in the prairie vole.

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CHAPTER 2 – Developmental trajectories and influences of environmental complexity on oxytocin receptor and vasopressin 1A receptor expression in male and female prairie voles

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ABSTRACT

Nonapeptide receptors, like oxytocin receptor (OTR) and vasopressin 1a receptor (V1aR), modulate a variety of functions across taxa, and mediate phenotypic variation within and between species. Despite the popularity of studying nonapeptides in adults, developmental perspectives on properties of OTR and V1aR expression are lacking. Study of prairie voles (*Microtus ochrogaster*) has facilitated an understanding of mechanisms of social behavior, and provides great potential to inform how early life experiences alter phenotype. We provide the first comprehensive profiling of OTR and V1aR in male and female prairie voles across postnatal development and into adulthood. Differences in receptor densities across the forebrain were region- and sex-specific. Postnatal changes in receptor expression fell into four themes: (1) constant over time, (2) increasing with age, (3) decreasing with age, or (4) peaking during late pre-weaning (postnatal day 15-21). We also examined the influence of post-weaning social and spatial enrichment (i.e., environmental complexity) on OTR and V1aR. Environmental complexity appeared to promote expression of OTR in males and females, and reduced expression of V1aR across several brain regions in males. Our results show that nonapeptide receptor profiles are plastic over development, and suggest that different patterns of expression might represent functional differences in sensitivity to nonapeptide activation over a period when social environments are dynamic. Our results on environmental complexity suggest that nonapeptide sensitivity responds flexibly to different environmental contexts during development. Understanding the developmental trajectories of nonapeptide receptors provides a better understanding of the dynamic nature of social behavior and the underlying mechanisms.

ABBREVIATIONS

AH: anterior hypothalamus

BLA: basolateral amygdala

BNST: bed nucleus of the stria terminalis

CeA: central amygdala

CP: caudate putamen

HPC: hippocampus

ICa: anterior insular cortex

ICm: medial insular cortex

ICp: posterior insular cortex

LDTh: laterodorsal thalamus

LS: lateral septum

NAcc: nucleus accumbens

MeA: medial amygdala

MDTh: mediodorsal thalamus

OBa: accessory olfactory bulbs

OBm: main olfactory bulbs

PFC: prefrontal cortex

PVN: paraventricular nucleus

RSC: retrosplenial cortex

SCN: suprachiasmic nucleus

SHi: septo-hippocampal nucleus

VMH: ventromedial hypothalamus

VPall: ventral pallidum

VPTh: ventroposterior thalamus

INTRODUCTION

Nonapeptide hormones, oxytocin (OT), vasopressin (VP) and their non-mammalian homologues, act as neuromodulators to support a suite of behavioral and regulatory functions throughout the forebrain (Albers, 2012; Borrow & Cameron, 2012; Wacker & Ludwig, 2012; K. A. Young, Gobrogge, Liu, & Wang, 2011; L. J. Young & Wang, 2004). Variation in the density of their receptors (oxytocin receptor, OTR and vasopressin receptor 1a, V1aR) introduces measurable differences that often translate into observable behavioral differences both within and between species (Albers, 2015; Beery, Lacey, & Francis, 2008; Dumais & Veenema, 2016). Therefore, characterizing the expression patterns of OTR and/or V1aR has informed much of our understanding of the natural variation in brain-behavior relationships, particularly in the area of social behavior.

The majority of the work in this area has focused on animals within a single age group. However, it is imperative to consider the dynamic action of nonapeptide systems across distinct developmental time-points. To the extent that the ontogeny of OTR and V1aR expression has been studied, the evidence indicates that OTR and V1aR can be dynamic in some regions of the forebrain but highly stable in others as animals age and mature (Hammock & Levitt, 2013; Olazabal & Alsina-Llanes, 2016; Shapiro & Insel, 1989; Tamborski, Mintz, & Caldwell, 2016; Tribollet, Charpak, Schmidt, Duboisdauphin, & Dreifuss, 1989; Tribollet, Goumaz, Raggenbass, Duboisdauphin, & Dreifuss, 1991; Wang & Young, 1997; Wang, Young, Liu, & Insel, 1997). Specifically, OTR or V1aR may be transiently high or low during infancy or juvenile life stages in some forebrain regions, whereas receptor expression remains constant throughout development into adulthood in other regions (reviewed in (Grinevich, Desarmenien, Chini,

Tauber, & Muscatelli, 2015; Hammock, 2015; Miller & Caldwell, 2015). These region-specific trajectories of OTR and V1aR development suggest that the influence of nonapeptides varies across developmental stages, with distributions of OTR and V1aR across the forebrain seeming to follow developmental programs. However, the majority of this work has focused on rats, with only a few studies in other species (including mice and voles). The literature across species shows some consistency, but more inconsistencies, which indicates that the nonapeptide system has responded to different selection pressures over the course of evolution (Goodson, Kelly, & Kingsbury, 2012; Kelly & Ophir, 2015; Ren et al., 2015). Indeed, species differences in these region-specific trajectories may be linked to concurrent socio-behavioral differences across development. A better understanding of the natural development of receptor expression has the potential to reveal the ways in which evolution has shaped various networks in the brain important for processing and mediating social behavior (Ketterson & Nolan, 1999).

The environment introduces a major source of variation on the nonapeptide system, and both social and ecological experiences can have profound influences on the expression and function of VP, OT, and their receptors in adulthood (Bales & Perkeybile, 2012; Carter, 2003; Cushing, 2013). For example, research across rodent species demonstrates the plastic response of nonapeptides systems to variation in early life social experiences (Bales & Perkeybile, 2012; Curley, Jensen, Mashoodh, & Champagne, 2011). Female prairie voles, *Microtus ochrogaster*, raised without fathers (i.e., single-mother rearing) exhibit increased OT mRNA expression in the PVN (Ahern & Young, 2009). Previous work from our lab demonstrates that male prairie voles experiencing single-mother rearing demonstrate greater V1aR expression in the RSC compared to males raised with both parents (Prounis, Foley, Rehman, & Ophir, 2015). In this same study, post-weaning

social environments also influenced OT and VP systems, with males living in isolation as juveniles developing greater OTR expression in several regions of the brain as adults (Prounis et al., 2015). The plasticity of OTR and V1aR that results from early life experiences presumably reflects, at least in part, the outcome of variation of endogenous OT and VP release and binding over development that ultimately impacts adult receptor phenotype. Indeed, early life OT manipulation in prairie voles influences the expression of adult species-typical behaviors, including pair bonding and alloparental care, in a sex-specific manner (Bales & Carter, 2003a; Bales, Pfeifer, & Carter, 2004). Early OT and VP manipulations also influence aggressive behavior differentially in adult males and females (Bales & Carter, 2003b; Stribley & Carter, 1999). Taken together, the evidence suggests that the natural development of OTR and V1aR is not only dynamic over the course of development, but that it is an open system that can be impacted by environmental context during development.

Over the past 25 years or more, prairie voles have served as an excellent species to understand the roles that nonapeptides play in mediating several rare and human-like behaviors, including bonding, monogamy, and bi-parental care. In this time, several studies have manipulated the early life experiences (behaviorally or pharmacologically; see above) in prairie voles to observe the consequences, yet we know relatively little about the natural patterns of OTR and V1aR over the course of development. Due to the prominence of the prairie vole in research on development and social behavior, and the importance of the nonapeptide system therein, the field is in need of a thorough developmental profile of OTR and V1aR expression in both male and female prairie voles, comparable to the work that has explored OTR and V1aR ontogeny in rats. Doing so will

also provide the ability to expand what we know about species differences among rodents in a meaningful way (Kelly and Ophir, 2015).

Here we conduct the most thorough analysis to date of the development of OTR and V1aR expression throughout the forebrain of both male and female prairie voles. We also investigated the ways in which social and spatial complexity experienced during post-weaning influences developmental trajectories of OTR and V1aR in each sex. We predicted that the patterns of OTR and V1aR over development in rats (and other species) will differ from many of the patterns observable in prairie voles, presumably reflecting the important socio-behavioral characteristics of this species (e.g., pair-bonding behavior, paternal care, and alloparental care) (Gobrogge & Wang, 2016). We also predicted that complex (and more naturalistic) social environments should produce differences in OTR and V1aR, which may relate to behavioral variation observed within the species (Okhovat, Berrio, Wallace, Ophir, & Phelps, 2015; Ophir, Campbell, Hanna, & Phelps, 2008; Ophir, Wolff, & Phelps, 2008; Perkeybile, Griffin, & Bales, 2013; Zheng, Larsson, Phelps, & Ophir, 2013). Expanding research on OTR and V1aR ontogeny across taxa, and on the influence of early life environments on this development, will advance our understanding of how nonapeptide systems might mediate species-typical behaviors across life stages.

METHODS

Pre-weaning and post-weaning housing conditions

We established 26 breeding pairs from F2 animals taken from our breeding colony, which was established using wild prairie voles we trapped in Champagne-Urbana, Illinois, USA. All breeders for this study were weaned at postnatal day (PND) 21, and separated into same-sex litters housed in standard polycarbonate cages (29 x 18 x 13 cm) lined with Sani-chip bedding

and provided nesting material. No animals in this experiment were raised in isolation. Water and rodent chow (Rodent Chow 5000, Harlan Teklad, Madison, WI, USA) were provided *ad libitum* and animals were maintained on a 14:10 hr light:dark cycle (lights on at 0600) with ambient temperature maintained at 20±2°C. All procedures were approved by the Institutional Animal Care and Use Committee.

After the animals reached sexual maturity (i.e., > PND 45) we paired males and females to create breeding pairs. To establish breeding pairs, we sexually primed females by adding a mixture of dirty bedding material and urine-soaked Sani-chips to the females' cages. This is a natural, robust, and non-invasive method of inducing sexual receptivity and estrus cycling in this species (Carter, Getz, Gavish, McDermott, & Arnold, 1980; Dluzen, Ramirez, Carter, & Getz, 1981; Richmond & Stehn, 1976). After 48 hours of exposing a female to these conditions, we introduced a male to each cage containing a female. Immediately after pairing, we monitored the animals for signs of overt aggression or potential harm. All pairs acclimated to the new housing conditions within 10 min, and the pairs were left alone to breed naturally. Twenty days after pairs were created, we began closely monitoring them for offspring; prairie vole gestation is approximately 21 days.

Pups from each breeding pair served as subjects for this study. We documented the birthday for each litter upon discovery of the litter. Litter size and sex ratios were also recorded for each litter. Pups from each litter were left alone until they reached one of seven ages: PND 6, 9, 12, 15, 18, 21, and 60. For animals assigned to pre-weaning groups (PND 6 – 21), we removed pups

from the home cage at the pre-assigned age and collected brains (see below). At least five males and five females were sampled for each age group (see below for final N-values).

We also created two groups of adult aged offspring (PND 60). Animals that were assigned to the PND 60 groups were weaned at 21 days, and housed with a same-sex sibling until they were 60 days old. One set of the PND 60 animals (N = 5 males, 9 females) was raised in standard housing conditions (as described above; with one same sex sibling in a standard shoebox cage). We refer to this group as 'Simple Adults' because they were provided relatively minimal social and environmental enrichment.

The second group of the PND 60 animals (N = 8 males, 8 females) was housed in cages exactly as those described above. However, each cage had a 7.6 cm hole in the side of the shoebox cage, which was connected to a four-sided clear Plexiglas arena (120cm x 120cm x 60cm) through a clear Plexiglas tunnel. The four sibling pairs that were housed in these 'satellite' cages were unrelated and unfamiliar to each other. The central arena was covered with alfalfa bedding, and contained PVC tubes for cover and a running wheel. Access to each 'satellite' cage was blocked with a wire cloth gate that prevented the pair from entering the arena. Every 24 h, we removed the wire gate for one of the satellite cages, allowing only the sibling pair in that cage access to the center arena. After 24 hours, the animals were returned to their satellite cage and the gate was replaced. The alfalfa was disrupted to remove any 'tunneling' in the arena and the running wheel was rotated 90° counterclockwise to the right. We then removed the gate for the next cage positioned to the counter clockwise adjacent wall. Although only one pair of siblings could freely enter the arena at any given moment, visual and olfactory contact at the wire cloth boundaries was possible when voles living in a blocked cage approached the blocked entryway.

We rotated access to each satellite cage each day from age 21 to age 60. Our rotation paradigm ensured high levels of olfactory complexity in the central chamber, with constantly changing patterns of urinary odors between each access window for a sibling pair. Although there were some factors we could not control using this design, our aim was to expose a juvenile vole to a spatially and socially complex environment that was more similar to what would be experienced in a natural habitat, at least when compared to standard laboratory housing. For these reasons, we referred to these animals as 'Enriched Adults'.

Tissue collection

As mentioned above, we collected brains from male and female pups at seven ages: PND 6, 9, 12, 15, 18, 21, and 60. Breeding pairs contributed no more than 1 male and 1 female to each age group, which were chosen at random from the litter. Immediately after extraction, we flash froze brains on powdered dry ice and wrapped them in aluminum foil, which was individually marked. All frozen brains were stored at -80 °C until cryosectioning. Later, we coronally sectioned brains at -18±2 °C to a thickness of 20 µm. Sections were mounted in four sets at 100 µm intervals on Superfrost Plus slides (Fisher Scientific Co., Pittsburgh, PA, USA). Each of four sets was then stored at -80 °C until they were used to visualize receptor density using autoradiography (see below).

OTR and V1aR autoradiography and figure preparation

We used our validated autoradiography protocol to visualize OTR and V1aR in subjects (see Ophir et al. 2013). We used ^{125}I-labeled radioligands to visualize oxytocin receptor (ornithine vasotocin analogue ([^{125}I]-OVTA); NEX254, PerkinElmer; Waltham, MA, USA) and

vasopressin receptor (vasopressin (Linear), V-1A antagonist (Phenylacetly1, O-Me-D-Tyr2, [^{125}I-Arg6]-); NEX310, PerkinElmer). We exposed radiolabelled tissue to film (GE Healthcare, Little Chalfont, UK) for 4 days. We assessed the relative density of ligand binding by inferring that receptor density relates to the optical density of exposed film. In this way, optical density measurements serve as a proxy for receptor density. We used ^{125}I-labelled radiographic standards (American Radiolabeled Chemicals, St Louis, MO, USA) to allow for conversion of optical density to receptor density. Films were digitized on a Microtek ArtixScan M1 (Microtek, Santa Fe Springs, CA, USA) at 1200 ppi with 8-bit gray-scale settings; contrast and brightness were uniformly (across all individuals) adjusted to maximize gray tones. Measurements of optical densities were collected using IMAGE-J (NIH, Bethesda, MD, USA). To assemble figures, the original digitized images were imported into Adobe Photoshop CS6 (v. 13.0 x64) where non-tissue backgrounds were removed and contrast and brightness were adjusted to minimize among-individual differences in nonspecific staining. Figures were assembled and labeled in PowerPoint (for Mac 2011, v. 14.7.3).

We calculated receptor density on each section by first converting optical density to disintegrations per minute (dpm), adjusted for tissue equivalence (TE; for 1 mg in the rat brain), by using a log function to fit curves generated by radiographic standards. To generate our measures of interest, we measured receptor optical density for each structure three times (once on a series of three brain sections, bilaterally). We also measured non-specific binding on each section by measuring the background levels collected (bilaterally) from fibrous areas that do not express either receptor on each of the same sections measured. The values for each structure

were averaged, converted to dpm/mg TE, and adjusted to represent specific binding by subtracting nonspecific binding from total binding for each area.

We assessed receptor density across the forebrain regions where OTR and V1aR are frequently and prominently expressed in prairie voles. Note that although several structures demonstrated different receptor expression levels as a function of age, no structures appeared to express receptors in adulthood but not at a young age, or vice versa. OTR was measured (rostral to caudal) in the PFC, ICa, NAcc, SHi, LS, CP, ICm, CeA, BLA, HPC, and ICp. V1aR was measured (rostral to caudal) in the OBm, OBa, VPall, LS, BNST, PVN, SCN, AH, LDTh, MDTh, VPTh, RSC, CeA, MeA, and VMH. All regions of interest were identified using the rat atlas (Paxinos & Watson, 2013); outlines of selected regions are depicted in the representative autoradiograms in Figs 2.1-2.9. Tissue damage during processing prevented scoring of particular regions in select individuals; final sample sizes for each region in each age group are reflected in Tables 2.1-2.4.

Data Analysis

We binned the pre-weaning aged animals into two groups for our analysis to capture the first half (PND 6, 9, 12; Early Pre-Wean) and second half (PND 15, 18, 21; Late Pre-Wean) of postnatal pre-weaning development. We chose to group our animals this way because these ages reflect specific behavioral and physiological milestones of prairie vole development (McGuire & Novak, 1984). Prairie voles just begin to enter the early stages of locomotion and independent behavior around PND 6-12, and become highly active, obtaining their own solid food, and engaging in play behavior by PND 15-21. Furthermore, these age-spans capture important

neurodevelopmental stages of growth and brain development in rodents and humans (Gottlieb, Keydar, & Epstein, 1977; Semple, Blomgren, Gimlin, Ferriero, & Noble-Haeusslein, 2013). Finally, our results demonstrated that nonapeptide receptor expression generally did not differ within these binned age groups (see Results and Tables 2.1-2.4 below).

As just explained, our study compared the influence of environmental and social enrichment on brain development. We operationally defined an effect of enrichment using at least one of the two following criteria. We considered enrichment to have impacted nonapeptide receptor neural phenotype either (1) when Enriched Adult animals were different from Late Pre-Wean animals (ages 15 to 21), but Simple Adult animals were not, or (2) when Simple and Enriched Adult values differed.

For practical reasons, male and female brains were labeled in separate autoradiography procedures, preventing direct comparison of expression values. We therefore limit discussion of sex differences to qualitative differences in patterns that are observed between the two sexes for any given region. We performed a nonparametric Kruskal-Wallis test, and the Dunn's test was used for *post hoc* pairwise multiple comparisons to compare receptor densities within each region across our four groups (Early Pre-Wean, Late Pre-Wean, Simple Adult, Enriched Adult). Nonparametric statistics were necessary because the assumption of homogeneity of variances between groups was not met, as determined by the Bartlett test. Dunn test *P*-values were rounded to nearest one-hundredth decimal, and we considered alpha ≤ 0.05 to be statistically significant.

Unfortunately, due to practical constraints, our sample sizes – particularly for the adult animal groups – were relatively small, placing us at risk of making type 2 statistical errors. Thus, although we did not consider such trends to be significantly different, in some instances non-statistically significant trends are worth mentioning for the readers consideration. Our intention in this paper is to shine a light on general patterns of receptor expression as a function of development and enrichment. Therefore, we include some discussion of non-statistically significant trends (1) to highlight the overarching patterns, (2) openly characterize those patterns and the specific data comparisons, and (3) provide the reader with such information within a relatively conservatively defined window in case type 2 errors were made. We limited our discussion of trends based on the following. Firstly, we calculated an estimated effect size of the average statistically different post hoc comparison for receptor expression between any group. That mean effect size (Cohen's d) was 1.36. We then used G*Power (v3.1.9.3; Faul et al. 2007, 2009) to estimate the necessary sample size per group needed to safely assess the contrasts. Based on these results, we only considered a non-significant trend to be at risk of being under powered and therefore worthy of discussion if the power analysis output indicated that an increase in sample size of one third or less was necessary; for example requiring an increase in sample size from 4 to 6.

RESULTS

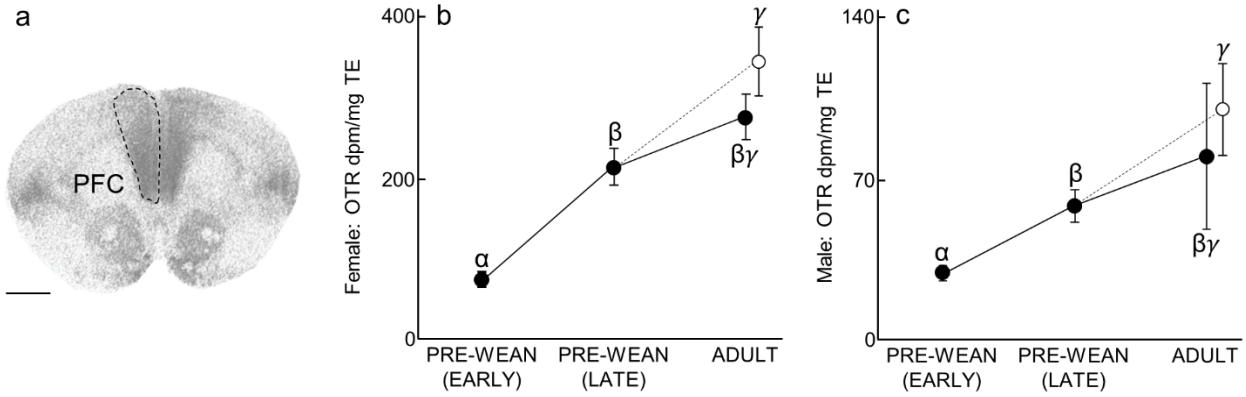
Patterns of Oxytocin Receptor Development

Patterns of developmental change in OTR densities across the forebrain were region-specific, and in some cases, sex-specific (Figs. 2.1-2.4, Table 2.1 and 2.3). In certain forebrain regions, expression profiles were stable and did not differ across development or as a function of

enrichment in adulthood. However, in many brain regions OTR density increased in expression over development, increased in expression due to enrichment, or both. Regions were analyzed individually and are reported separately below.

Prefrontal Cortex (PFC): Both males ($x^2(3) = 26.77$, $P < 0.0001$; Fig 2.1C, Table 2.1) and females ($x^2(3) = 34.25$, $P < 0.0001$; Fig 2.1B, Table 2.3) showed increases in PFC OTR expression over development and demonstrated increases in PFC OTR as a result of enrichment. Post hoc tests showed that PFC OTR expression in Late Pre-Wean males, Simple Adult males, and Enriched Adult males was significantly greater than in Early Pre-Wean males (Dunn's test; $P = 0.0002$, $P = 0.007$ and $P < 0.0001$, respectively), and Enriched Adult males had significantly more PFC OTR than Late Pre-Wean males ($P = 0.04$). Late Pre-Wean females, Simple Adult females, and Enriched Adult females showed significantly more PFC OTR than Early Pre-Wean females (all $P < 0.0001$), and Enriched Adult females had significantly more PFC OTR than Late Pre-Wean females (all $P < 0.0001$); Enriched Adult females had significantly more PFC OTR than Late Pre-Wean females ($P = 0.05$). No differences in PFC OTR were found in the remaining group comparisons (***Male PFC OTR***: Late Pre-Wean vs Simple Adult: $P = 0.48$, Simple Adult vs Enriched Adult: $P = 0.10$; ***Female PFC OTR:*** Late Pre-Wean vs Simple Adult: $P = 0.17$, Simple Adult vs Enriched Adult: $P = 0.32$).

Figure 2.1. Prefrontal cortex (PFC) oxytocin receptor (OTR) expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical OTR binding in the PFC of an adult. Dashed line encircles the PFC to indicate the borders of this region. Scale bar = 1 mm. **(B)** Mean (±SEM) PFC OTR expression (dpm/mg TE) in females at early pre-wean (PND 6, 9, 12), late pre-wean (PND 15, 18, 21), and adulthood (PND 60). Adults living in simple (solid black line) and enriched (dashed line) environments are overlaid. **(C)** Mean (±SEM) PFC OTR expression (dpm/mg TE) in males, following panel B. Greek characters (α, β, and γ) denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups; bars that do not share a character were significantly different.

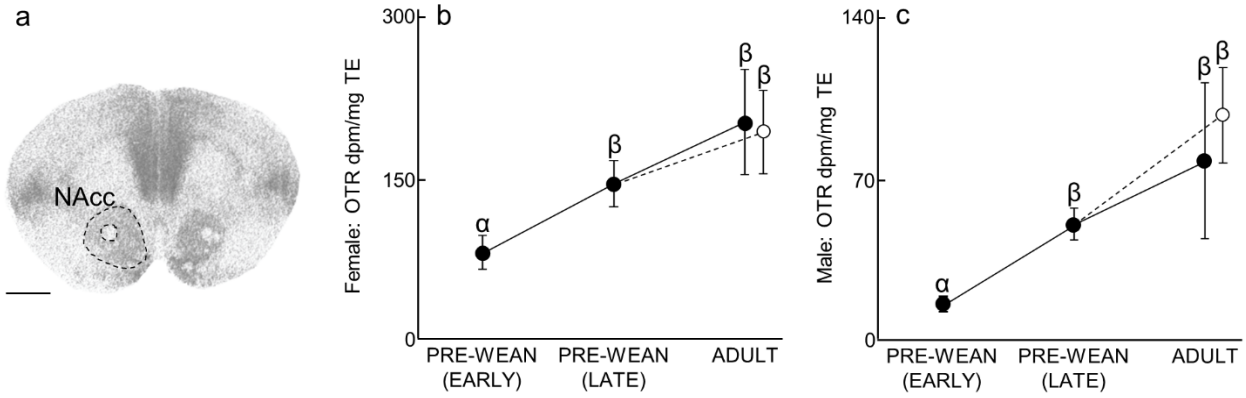


Insular Cortex, anterior (ICa): Both males ($x^2(3) = 9.76$, $P = 0.02$; Table 2.1) and females ($x^2(3) = 12$, $P = 0.005$; Table 2.3) demonstrated significant increases in ICa OTR as a result of enrichment, but only females demonstrated significant increases over development. ICa OTR expression in Enriched Adult males was significantly greater than in all other male groups (Early Pre-Wean: $P = 0.003$; Late Pre-Wean: $P = 0.005$; Simple Adult: $P = 0.005$). A similar pattern was found in females, with significantly greater ICa OTR expression in Enriched Adult females than in Early Pre-Wean females and Late Pre-Wean females ($P = 0.0003$ and $P = 0.04$, respectively). Simple Adult females and Late Pre-Wean females also had higher expression than Early Pre-Wean females ($P = 0.04$ and $P = 0.02$, respectively). No differences in ICa OTR were found in the remaining group comparisons (***Male ICa OTR:*** Early Pre-Wean vs Late Pre-Wean:

$P = 0.49$, Early Pre-Wean vs Simple Adult: $P = 0.25$, Late Pre-Wean vs Simple Adult: $P = 0.25$; ***Female ICa OTR:*** Late Pre-Wean vs Simple Adult: $P = 0.37$, Simple Adult vs Enriched Adult: $P = 0.13$).

Nucleus Accumbens (NAcc): Both males ($x^2(3) = 24.32$, $P < 0.0001$; Fig 2.2C, Table 2.1) and females ($x^2(3) = 12.04$, $P = 0.007$; Fig 2.2B, Table 2.3) demonstrated significant increases in NAcc OTR across development. NAcc OTR expression in Late Pre-Wean males, Simple Adult males, and Enriched males was significantly greater than in Early Pre-Wean males ($P = 0.0001$, $P = 0.001$, and $P < 0.0001$, respectively). NAcc OTR among females showed the same pattern: NAcc OTR expression in Late Pre-Wean females, Simple Adult females, and Enriched females was significantly greater than in Early Pre-Wean females ($P = 0.01$, $P = 0.007$, and $P = 0.003$, respectively). No differences in NAcc OTR were found in the remaining group comparisons (***Male NAcc OTR:*** Late Pre-Wean vs Simple Adult: $P = 0.30$, Late Pre-Wean vs Enriched Adult: $P = 0.13$, Simple Adult vs Enriched Adult: $P = 0.35$; ***Female NAcc OTR:*** Late Pre-Wean vs Simple Adult: $P = 0.23$, Late Pre-Wean vs Enriched Adult: $P = 0.19$, Simple Adult vs Enriched Adult: $P = 0.49$).

Figure 2.2. Nucleus accumbens (NAcc) OTR expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical OTR binding in the NAcc of an adult. Dashed line encircles the NAcc to indicate the borders of this region. Scale bar = 1 mm. **(B-C)** Mean (±SEM) NAcc OTR (dpm/mg TE) for females and males, respectively, following Figure 2.1. Greek characters (α, β, and γ) denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups; bars that do not share a character were significantly different.



Caudate-Putamen (CP): Both males ($x^2(3) = 18.38$, $P = 0.0004$; Table 2.1) and females ($x^2(3) = 15.76$, $P = 0.001$; Table 2.3) demonstrated significant increases in CP OTR over development, but only males demonstrated a significant increase in CP OTR as a result of enrichment. CP OTR expression in Late Pre-Wean males, Simple Adult males, and Enriched Adult males was significantly higher than in Early Pre-Wean males ($P = 0.004$, $P = 0.03$, and $P < 0.0001$, respectively). Enriched Adult males also had significantly higher CP OTR expression than Late Pre-Wean males ($P = 0.03$). CP OTR expression in Simple Adult females and Enriched Adult females was significantly higher than in Early Pre-Wean females ($P = 0.004$ and $P = 0.0002$, respectively). Further, Enriched Adult females had significantly higher CP OTR expression than Late Pre-Wean females ($P = 0.02$). Two non-significant trends were also found in females, suggesting that Late Pre-Wean females had higher CP OTR expression than Early Pre-Wean females ($P = 0.06$) and Simple Adult females had higher CP OTR expression than Late Pre-

Wean females (P = 0.07). No differences in CP OTR were found in the remaining group comparisons (***Male CP OTR:*** Late Pre-Wean vs Simple Adult: P = 0.47, Simple Adult vs Enriched Adult: P = 0.09; ***Female CP OTR:*** Simple Adult vs Enriched Adult: P = 0.34).

Insular Cortex, medial (ICm): An increase in ICm OTR across development was found in females only ($x^2(3) = 15.59$, P = 0.001; Table 2.3). ICm OTR expression in Late Pre-Wean females, Simple Adult females, and Enriched Adult females was significantly higher than in Early Pre-Wean females (P = 0.04, P = 0.0009, and P = 0.0008, respectively). Further, ICm OTR expression in Simple Adult females and Enriched Adult females was significantly higher than in Late Pre-Wean females (P = 0.04 and P = 0.05, respectively). No differences in ICm OTR were found in the remaining group comparisons (***Female ICm OTR:*** Simple Adult vs Enriched Adult: P = 0.41). In males, ICm OTR was stable across development and did not differ ($x^2(3) = 1.41$, P = 0.70; Table 2.1).

Central Amygdala (CeA): Both males ($x^2(3) = 19.19$, P = 0.0003; Table 2.1) and females ($x^2(3) = 18.13$, P = 0.0004; Table 2.3) demonstrated significant increases in CeA OTR over development. Furthermore, CeA OTR in females demonstrated significant increases in CeA OTR as a result of enrichment. CeA OTR expression in Late Pre-Wean, Simple Adult males, and Enriched Adult males was greater than in Early Pre-Wean males (P = 0.0004, P = 0.05, and P = 0.0001, respectively). CeA OTR expression in Late Pre-Wean females, Simple Adult females, and Enriched Adult females was significantly higher than in Early Pre-Wean females (P = 0.03, P = 0.006, and $P < 0.0001$, respectively). CeA OTR expression in Enriched Adult females was also significantly higher than in Late Pre-Wean females (P = 0.009). No differences in CeA OTR

were found in the remaining group comparisons (***Male CeA OTR:*** Late Pre-Wean vs Simple Adult: $P = 0.26$, Late Pre-Wean vs Enriched Adult: $P = 0.14$, Enriched Adult vs Simple Adult males $P = 0.08$; ***Female CeA OTR:*** Late Pre-Wean vs Simple Adult: $P = 0.13$, Simple Adult vs Enriched Adult: $P = 0.17$).

Basolateral Amygdala (BLA): Both males ($x^2(3) = 19.05$, $P = 0.0003$; Table 2.1) and females ($x^2(3) = 14.29$, $P = 0.003$; Table 2.3) showed significant increases in BLA OTR expression as a result of enrichment. BLA OTR significantly increased in females over development, whereas males demonstrated a non-significant trend following the same pattern. BLA OTR expression in Late Pre-Wean males and Enriched Adult males was significantly higher than in Early Pre-Wean males ($P = 0.002$ and $P < 0.0001$, respectively). Enriched Adult males also had significantly higher BLA OTR expression than Late Pre-Wean males and Simple Adult males (both *P's* = 0.04). A non-significant trend was also found in males, suggesting Simple Adult males have higher BLA OTR than Early Pre-Wean males ($P = 0.07$). BLA OTR expression in Late Pre-Wean females, Simple Adult females, and Enriched Adult females was significantly higher than in Early Pre-Wean females ($P = 0.03$, $P = 0.01$, and $P = 0.0003$, respectively). Moreover, BLA OTR expression in Enriched Adult females was significantly higher than in Late Pre-Wean females ($P = 0.03$). No differences in BLA OTR were found in the remaining group comparisons (***Male BLA OTR:*** Late Pre-Wean vs Simple Adult: $P = 0.33$; ***Female BLA OTR:*** Late Pre-Wean vs Simple Adult: $P = 0.19$, Simple Adult vs Enriched Adult: $P = 0.24$).

Septohippocampal Nucleus (SHi), Lateral Septum (LS), Hippocampus (HPC), and Insular Cortex, posterior (ICp): In both males and females, OTR expression in the SHi (males: $x^2(3) = 5.48$, $P = 0.14$; females: $x^2(3) = 5.63$, $P = 0.13$; Table 2.1 and 2.3), LS (males: $x^2(3) = 5.02$, $P =$

0.17; females: $x^2(3) = 5.08$, $P = 0.17$; Fig 2.3, Table 2.1 and 2.3), HPC (males: $x^2(3) = 2.35$, $P = 0.50$; females: $x^2(3) = 4.38$, $P = 0.22$; Fig 2.4, Table 2.1 and 2.3), and ICp (males: $x^2(3) = 2.19$, $P = 0.53$; females: $x^2(3) = 5.69$, $P = 0.13$; Table 2.1 and 2.3) was stable across development and showed no statistical differences.

Figure 2.3. Lateral septum (LS) OTR expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical OTR binding in the LS of an adult. Dashed line encircles the LS to indicate the borders of this region. Scale bar = 1 mm. **(B-C)** Mean (±SEM) LS OTR (dpm/mg TE) for females and males, respectively, following Figure 2.1.

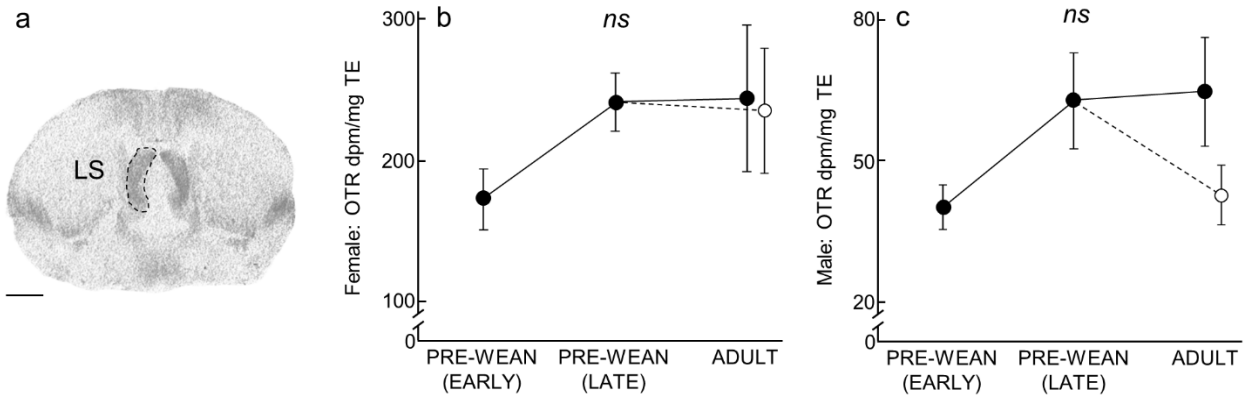
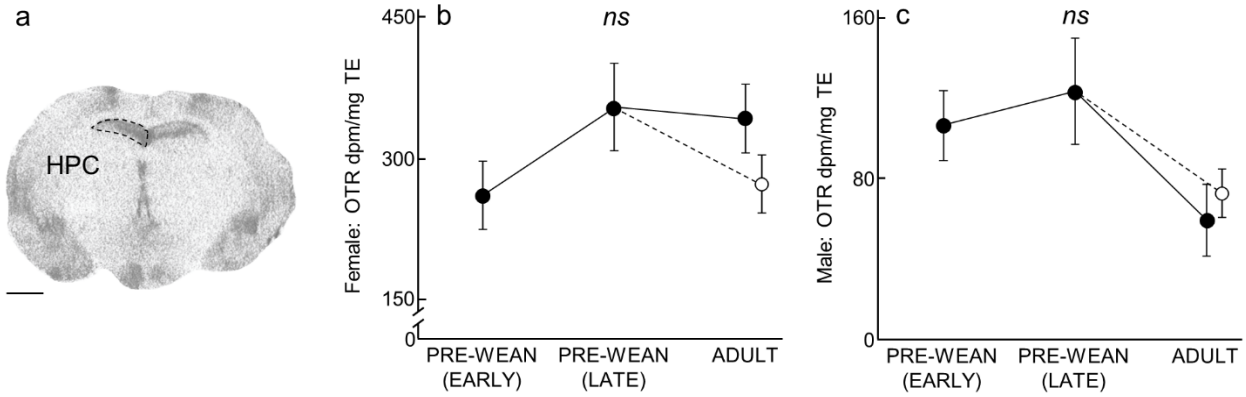


Figure 2.4. Hippocampus (HPC) OTR expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical OTR binding in the HPC of an adult. Dashed line encircles the HPC to indicate the borders of this region. Scale bar = 1 mm. **(B-C)** Mean (±SEM) HPC OTR (dpm/mg TE) for females and males, respectively, following Figure 2.1.



Patterns of Vasopressin Receptor Development

Patterns of developmental change in V1aR densities across the forebrain were region-specific, and in some cases, sex-specific (Figs. 2.5-2.9, Table 2.2 and 2.4). In certain forebrain regions, expression profiles were stable and did not differ across development or as a function of enrichment in adulthood. However, V1aR density was dynamic over development, was influenced by enrichment, or both in most brain regions we investigated. Compared to OTR regions, patterns of V1aR developmental trajectories were more variable, and included regions where peak V1aR expression occurred during Early Pre-Wean and/or Late Pre-Wean. The influences of enrichment on V1aR were also nearly exclusively seen in males. Generally, enrichment was associated with less V1aR when compared to the other groups. Regions were analyzed individually and are reported separately below.

Main Olfactory Bulbs (OBm): Both males ($x^2(3) = 29.2$, $P < 0.0001$; Table 2.2) and females ($x^2(3) = 21.9$, $P < 0.0001$; Table 2.4) showed increases in OBm V1aR expression over development. OBm V1aR expression in Late Pre-Wean males, Simple Adult males, and Enriched Adult males was significantly higher than in Early Pre-Wean males (Dunn's test: $P = 0.003$, $P = 0.0002$, and $P < 0.0001$, respectively). Further, Simple Adult males and Enriched Adult males had significantly higher OBm V1aR expression than Late Pre-Wean males ($P = 0.04$ and $P = 0.005$, respectively). OBm V1aR expression in Late Pre-Wean females, Simple Adult females, and Enriched Adult females was significantly higher than in Early Pre-Wean females ($P = 0.008$, $P = 0.0002$, and $P < 0.0001$, respectively). Furthermore, OBm V1aR expression in Enriched Adult females was significantly higher than in Late Pre-Wean females ($P = 0.02$). A non-significant trend was also found in females, suggesting Simple Adult females might have

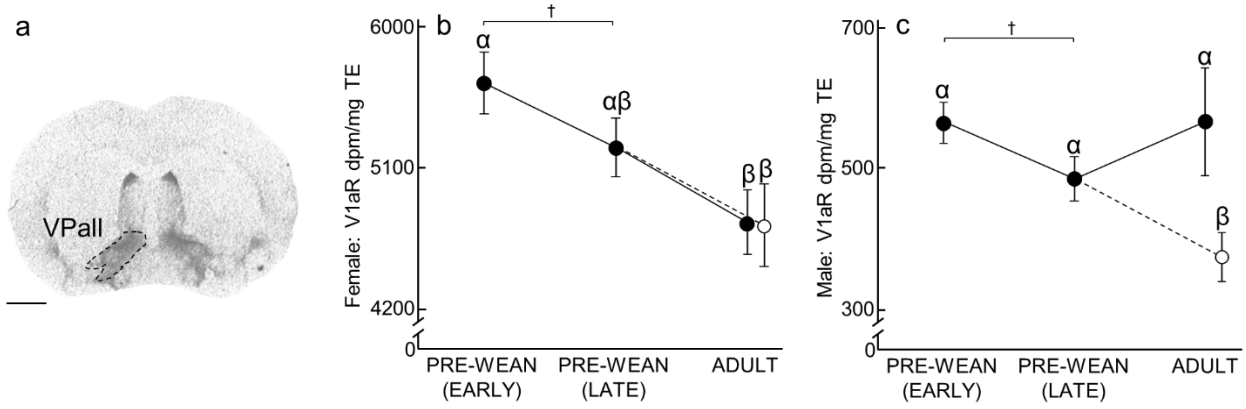
higher OBm V1aR expression than Late Pre-Wean females ($P = 0.07$). No differences in OBm V1aR were found in the remaining group comparisons (***Male OBm V1aR:*** Simple Adult vs Enriched Adult: $P = 0.39$; ***Female OBm V1aR:*** Simple Adult vs Enriched Adult: $P = 0.29$).

Accessory Olfactory Bulbs (OBa): Both males ($x^2(3) = 30.95$, $P < 0.0001$; Table 2.2) and females ($x^2(3) = 26.07$, $P < 0.0001$; Table 2.4) showed significant increases in OBa V1aR expression over development. OBa V1aR expression in Late Pre-Wean males, Simple Adult males, and Enriched Adult males was significantly higher than in Early Pre-Wean males ($P = 0.0001$, $P = 0.0001$, and $P < 0.0001$, respectively). OBa V1aR expression in Enriched Adult males was significantly higher than in Late Pre-Wean males ($P = 0.04$). A non-significant trend was also found in males, suggesting Simple Adult males might have higher OBa V1aR expression than Late Pre-Wean males ($P = 0.07$). OBa expression in Late Pre-Wean females, Simple Adult females, and Enriched Adult females was significantly higher than in Early Pre-Wean females ($P < 0.0001$, $P = 0.0004$, and $P < 0.0001$, respectively). No differences in OBa V1aR were found in the remaining group comparisons (***Male OBa V1aR:*** Simple Adult vs Enriched Adult: $P = 0.47$; ***Female OBa V1aR:*** Late Pre-Wean vs Simple Adult: $P = 0.49$, Late Pre-Wean vs Enriched Adult: $P = 0.15$, Simple Adult vs Enriched Adult: $P = 0.17$).

Ventral Pallidum (VPall): Males demonstrated significantly less VPall V1aR expression as a result of enrichment ($x^2(3) = 12.68$, $P = 0.01$; Fig 2.5C, Table 2.2), whereas females demonstrated a significant decrease in VPall V1aR expression over development ($x^2(3) = 10.67$, $P = 0.01$; Fig 2.5B, Table 2.4). VPall V1aR expression in Enriched Adult males was significantly lower than in Early Pre-Wean males, Late Pre-Wean males, and Simple Adult

males ($P = 0.0005$, $P = 0.02$, and $P < 0.003$, respectively). A non-significant trend was also found in males, suggesting Late Pre-Wean males have lower VPall V1aR expression than Early Pre-Wean males ($P = 0.07$). VPall V1aR expression in Simple Adult females and Enriched Adult females was significantly lower than in Early Pre-Wean females ($P = 0.003$ and $P = 0.005$, respectively). A non-significant trend was also found in females, suggesting that expression for Late Pre-Wean females might be lower than Early Pre-Wean females ($P = 0.07$). No differences in VPall V1aR were found in the remaining group comparisons (***Male VPall V1aR:*** Early Pre-Wean vs Simple Adult: $P = 0.36$, Late Pre-Wean vs Simple Adult: $P = 0.09$; ***Female VPall V1aR:*** Late Pre-Wean vs Simple Adult: $P = 0.082$, Late Pre-Wean vs Enriched Adult: $P = 0.09$, Simple Adult vs Enriched Adult: $P = 0.50$).

Figure 2.5. Ventral pallidum (VPall) V1aR expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical V1aR binding in the VPall of an adult. Dashed line encircles the VPall to indicate the borders of this region. Scale bar = 1 mm. **(B-C)** Mean (±SEM) VPall V1aR (dpm/mg TE) for females and males, respectively, following Figure 2.1. Greek characters (α, β, and γ) denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups; bars that do not share a character were significantly different. † denotes non-significant trends ($P < 0.07$) between groups.



Bed Nucleus of the Stria Terminalis (BNST): Males showed significantly less BNST V1aR expression as a result of enrichment ($x^2(3) = 14.18$, $P = 0.003$; Table 2.2). Additionally, a non-significant trend in males suggest that BNST V1aR expression decreases over development. BNST V1aR expression in Late Pre-Wean males was significantly lower than in Early Pre-Wean males ($P = 0.04$). Enriched Adult males also had less BNST V1aR expression than Early Pre-Wean males and Late Pre-Wean males ($P = 0.0001$ and $P = 0.01$, respectively). A non-significant trend was found in males, suggesting that Simple Adult males might have lower BNST V1aR expression than Early Pre-Wean males ($P = 0.07$). No differences in BNST V1aR were found in the remaining group comparisons (***Male BNST V1aR:*** Late Pre-Wean vs Simple Adult: $P = 0.37$, Enriched Adult vs Simple Adult: $P = 0.08$). In females, BNST V1aR was stable across development and showed no statistical differences ($x^2(3) = 2.88$, $P = 0.41$; Table 2.4).

Paraventricular Nucleus (PVN): Both males ($x^2(3) = 22.46$, $P < 0.0001$; Table 2.2) and females ($x^2(3) = 18.40$, $P < 0.0004$; Table 2.4) showed a decrease in PVN V1aR over development, and only females showed an apparent decrease in PVN V1aR as a result of enrichment. PVN V1aR expression in Late Pre-Wean males, Simple Adult males, and Enriched Adult males was significantly lower than in Early Pre-Wean males ($P = 0.05$, $P = 0.005$, and $P < 0.0001$, respectively). Further, PVN V1aR expression in Enriched Adult males was significantly lower than in Late Pre-Wean males ($P = 0.001$). A non-significant trend was also found in males, suggesting that Simple Adult males might have lower PVN V1aR expression than Late Pre-Wean males ($P = 0.07$). PVN V1aR expression in Late Pre-Weaning females and Simple Adult females were significantly lower than in Early Pre-Wean females ($P = 0.01$, $P = 0.0006$, and $P = 0.0001$, respectively). Furthermore, PVN V1aR expression in Enriched Adult females was significantly lower than in Late Pre-Wean females ($P = 0.03$). No differences in PVN V1aR were

found in the remaining group comparisons (***Male PVN V1aR:*** Simple Adult vs Enriched Adult: $P = 0.16$; ***Female PVN V1aR:*** Late Pre-Wean vs Simple Adult: $P = 0.10$, Simple Adult vs Enriched Adult: $P = 0.29$).

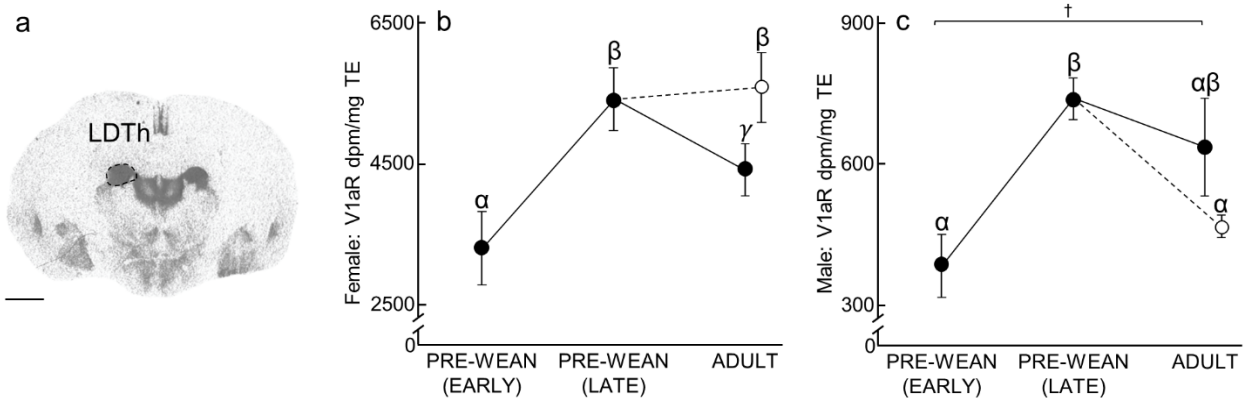
Suprachiasmic Nucleus (SCN): Males showed a decrease in SCN V1aR over development and as a result of enrichment ($x^2(3) = 20.5$, $P = 0.0001$; Table 2.2). SCN V1aR expression in Late Pre-Wean males, Simple Adult males, and Enriched Adult males was significantly lower than in Early Pre-Wean males ($P = 0.02$, $P = 0.005$, and $P < 0.0001$, respectively). Further, SCN V1aR expression in Enriched Adult males was significantly lower than in Late Pre-Wean males and in Simple Adult males ($P = 0.003$ and $P = 0.05$). No differences in SCN V1aR were found in the remaining group comparisons (***Male SCN V1aR:*** Late Pre-Wean vs Simple Adult: $P = 0.29$). In females, SCN V1aR was stable across development and showed no statistical differences ($x^2(3) = 3.59$, $P = 0.31$; Table 2.4).

Anterior Hypothalamus (AH): Both males ($x^2(3) = 21.87$, $P < 0.0001$; Table 2.2) and females ($x^2(3) = 17.58$, $P = 0.0005$; Table 2.4) showed a decrease in AH V1aR expression over development. AH V1aR expression in Simple Adult males and Enriched Adult males was significantly lower than in Early Pre-Wean males ($P = 0.0007$ and $P < 0.0001$, respectively) and in Late Pre-Wean males ($P = 0.006$ and $P = 0.001$, respectively). AH V1aR expression in Late Pre-Wean females, Simple Adult females, and Enriched Adult females was significantly lower than in Early Pre-Wean females ($P = 0.04$, $P = 0.0001$, and $P = 0.001$, respectively). Further, AH V1aR expression in Simple Adult females and Enriched Adult females was significantly lower than in Late Pre-Wean females ($P = 0.02$ and $P = 0.05$, respectively). No differences in AH

V1aR were found in the remaining group comparisons (***Male AH V1AR:*** Early Pre-Wean vs Late Pre-Wean: $P = 0.18$, Simple Adult vs Enriched Adult: $P = 0.46$; ***Female AH V1AR:*** Simple Adult vs Enriched Adult: $P = 0.35$).

Laterodorsal Thalamus (LDTh): Females ($x^2(3) = 14.75$, $P = 0.002$; Fig 2.6B, Table 2.4) showed a peak of LDTh V1aR expression during Late Pre-Weaning, but also a significant increase in expression over the course of development. Males demonstrated a non-significant trend toward increasing V1aR expression over development ($x^2(3) = 16.56$, $P = 0.0009$; Fig 2.6C, Table 2.2). Enrichment, however, appeared to produce an increase of LDTh V1aR expression in females, and a decrease of LDTh V1aR expression in males. LDTh V1aR expression in Late Pre-Wean males was significantly greater than in Early Pre-Wean males and Enriched Adult males ($P = 0.0001$ and $P = 0.003$, respectively). A non-significant trend was also found in males, suggesting that LDTh V1aR expression might be higher in Simple Adult males than in Early Pre-Wean males ($P = 0.07$). LDTh V1aR expression in Late Pre-Wean females and Enriched Adult females was significantly greater than in Early Pre-Wean females ($P = 0.0009$ and $P = 0.001$, respectively) and Simple Adult females ($P = 0.03$ and $P = 0.02$, respectively). No differences in LDTh V1aR were found in the remaining group comparisons (***Male LDTh V1aR:*** Early Pre-Wean vs Enriched Adult: $P = 0.39$, Late Pre-Wean vs Simple Adult: $P = 0.19$, Simple Adult vs Enriched Adult: $P = 0.12$; ***Female LDTh V1aR:*** Early Pre-Wean vs Simple Adult: $P = 0.28$, Late Pre-Wean vs Enriched Adult: $P = 0.32$).

Figure 2.6. Laterodorsal thalamus (LDTh) V1aR expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical V1aR binding in the LDTh of an adult. Dashed line encircles the LDTh to indicate the borders of this region. Scale bar = 1 mm. **(B-C)** Mean (±SEM) LDTh V1aR (dpm/mg TE) for females and males, respectively, following Figure 2.1. Greek characters (α, β, and γ) denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups; bars that do not share a character were significantly different. † denotes a non-significant trend ($P < 0.07$) between groups.



Mediodorsal Thalamus (MDTh): Both males ($x^2(3) = 21.01$, $P = 0.0001$; Table 2.2) and females ($x^2(3) = 13.9$, $P = 0.003$; Table 2.4) showed an increase in MDTh V1aR expression over development. In contrast, enrichment appeared to produce a decrease in MDTh V1aR expression in males only. MDTh V1aR expression in Late Pre-Wean males and Simple Adult males was significantly greater than in Early Pre-Wean males ($P < 0.0001$ and $P = 0.01$, respectively). MDTh V1aR expression in Enriched Adult males was significantly less than in Late Pre-Wean males ($P = 0.009$). MDTh V1aR expression in Late Pre-Wean females, Simple Adult females, and Enriched Adult females was significantly greater than in Early Pre-Wean females ($P = 0.0005$, $P = 0.03$, and $P = 0.002$, respectively). No differences in MDTh_V1aR were found in the remaining group comparisons (***Male MDTh V1aR:*** Early Pre-Wean vs Enriched Adult: $P = 0.13$,

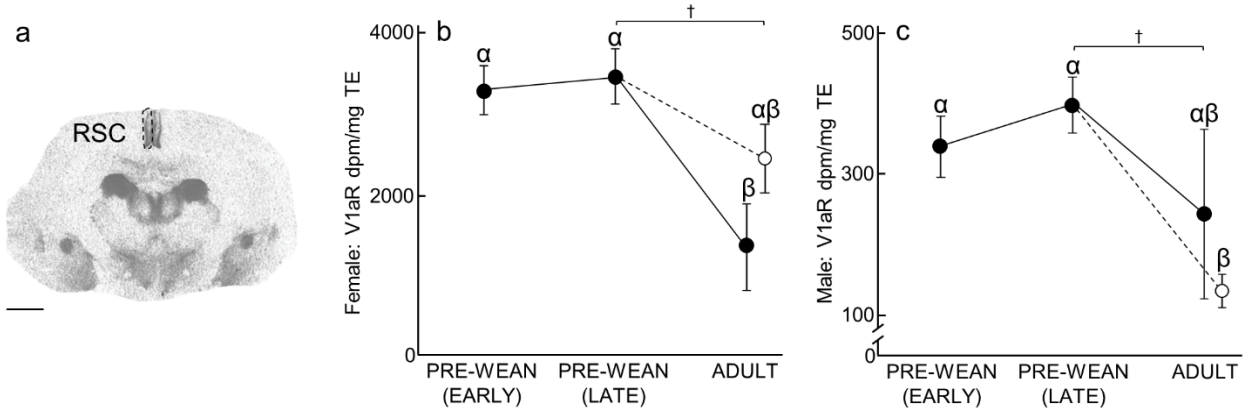
Late Pre-Wean vs Simple Adult: $P = 0.30$, Simple Adult vs Enriched Adult: $P = 0.11$; ***Female MDTh V1aR:*** Late Pre-Wean vs Simple Adult: $P = 0.19$, Late Pre-Wean vs Enriched Adult: $P = 0.44$, Simple Adult vs Enriched Adult: $P = 0.18$).

Ventroposterior Thalamus (VPTh): Both males ($x^2(3) = 13.04$, $P = 0.005$; Table 2.2) and females ($x^2(3) = 12.92$, $P = 0.005$; Table 2.4) showed a peak of VPTh V1aR expression during Late Pre-Weaning. VPTh V1aR expression in Late Pre-Wean males was significantly greater than in Early Pre-Wean males, Simple Adult males, and Enriched Adult males ($P = 0.01$, $P = 0.04$, and $P = 0.0003$, respectively). VPTh V1aR expression in Enriched Adult males was significantly less than in Early Pre-Wean males ($P = 0.04$). In females, VPTh V1aR expression was significantly greater in Late Pre-Wean females than in Early Pre-Wean females, Simple Adult females, and Enriched Adult females ($P = 0.0003$, $P = 0.006$, and $P = 0.05$, respectively). No differences in VPTh V1aR were found in the remaining group comparisons (***Male VPTh V1aR:*** Early Pre-Wean vs Simple Adult: $P = 0.34$, Simple Adult vs Enriched Adult: $P = 0.19$; ***Female VPTh V1aR:*** Early Pre-Wean vs Simple Adult: $P = 0.39$, Early Pre-Wean vs Enriched Adult: $P = 0.12$, Simple Adult vs Enriched Adult: $P = 0.22$).

Retrosplenial Cortex (RSC): RSC V1aR expression for both males and females differed across treatments (males: $x^2(3) = 13.28$, $P = 0.004$; females ($x^2(3) = 11.58$, $P = 0.009$; Fig 2.7B-C, Table 2.2 and 2.4). Post hoc analyses revealed that the pattern of receptor expression progressively decreased over development for both sexes, but only females showed a significant difference over development. Specifically, Simple Adult males tended to express less RSC V1aR than Late Pre-Wean males ($P = 0.07$), whereas RSC V1aR expression in Simple Adult females

was significantly less than in Early Pre-Wean females and Late Pre-Wean females ($P = 0.002$ and $P = 0.001$, respectively). RSC V1aR expression in Enriched Adult males was significantly less than in Early Pre-Wean males and Late Pre-Wean males ($P = 0.003$ and $P = 0.0002$, respectively). Moreover, Enriched Adult females tended to express less RSC V1aR than Late Pre-Wean females ($P = 0.08$). No differences in RSC V1aR were found in the remaining group comparisons (***Male RSC V1aR:*** Early Pre-Wean vs Late Pre-Wean: $P = 0.15$, Early Pre-Wean vs Simple Adult: $P = 0.19$, Simple Adult vs Enriched Adult: $P = 0.13$; ***Female RSC V1aR:*** Early Pre-Wean vs Late Pre-Wean: $P = 0.45$, Early Pre-Wean vs Enriched Adult: $P = 0.09$, Simple Adult vs Enriched Adult: $P = 0.09$).

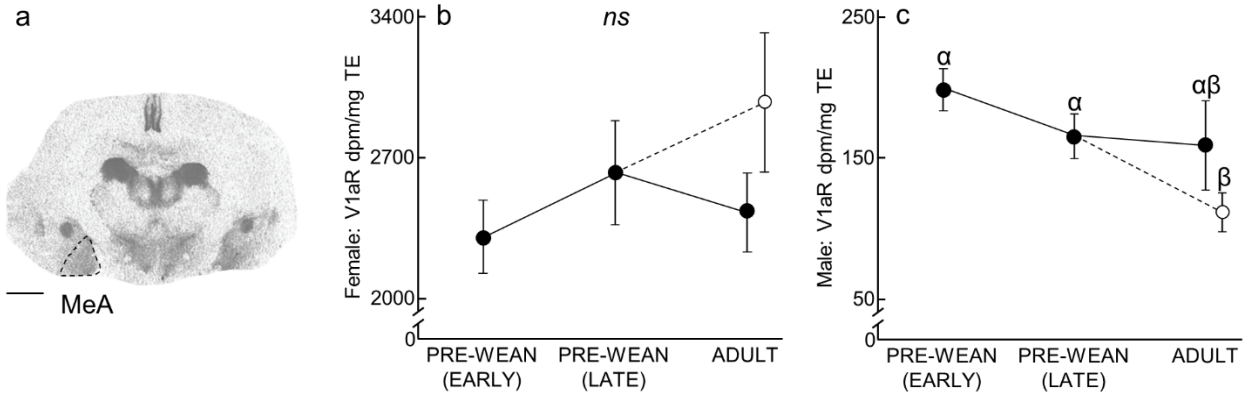
Figure 2.7. Retrosplenial cortex (RSC) V1aR expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical V1aR binding in the RSC of an adult. Dashed line encircles the RSC to indicate the borders of this region. Scale bar = 1 mm. **(B-C)** Mean (±SEM) RSC V1aR (dpm/mg TE) for females and males, respectively, following Figure 2.1. Greek characters (α, β, and γ) denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups; bars that do not share a character were significantly different. † denotes non-significant trends ($P < 0.08$) between



groups.

Medial Amygdala (MeA): Males showed a decrease in MeA V1aR due to enrichment ($x^2(3) = 9.84$, $P = 0.02$; Fig 2.8C, Table 2.2). MeA V1aR expression in Enriched Adult males was significantly less than in Early Pre-Wean males and Late Pre-Wean males ($P = 0.0009$ and $P = 0.02$, respectively). No differences in MeA V1aR were found in the remaining group comparisons (***Male MeA V1aR:*** Early Pre-Wean vs Late Pre-Wean: $P = 0.10$, Early Pre-Wean vs Simple Adult: $P = 0.13$, Late Pre-Wean vs Simple Adult: $P = 0.38$, Simple Adult vs Enriched Adult: $P = 0.13$). In females, MeA V1aR was stable across development and showed no significant differences ($x^2(3) = 3.71$, $P = 0.30$; Fig 2.8B, Table 2.4).

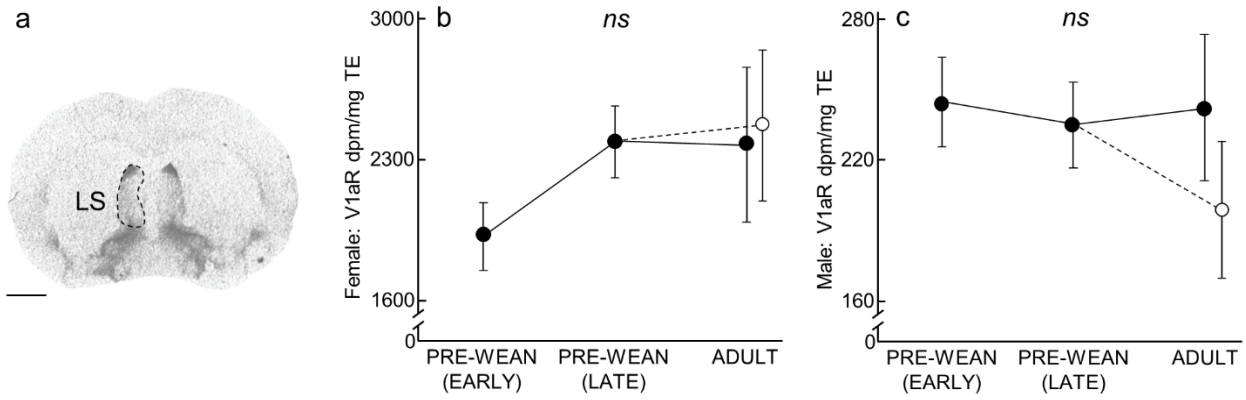
Figure 2.8. Medial amygdala (MeA) V1aR expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical V1aR binding in the MeA of an adult. Dashed line encircles the MeA to indicate the borders of this region. Scale bar = 1 mm. **(B-C)** Mean (±SEM) MeA V1aR (dpm/mg TE) for females and males, respectively, following Figure 2.1. Greek characters (α, β, and γ) denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups; bars that do not share a character were significantly different.



Ventromedial Hypothalamus (VMH): Males showed a decrease in VMH V1aR due to enrichment ($x^2(3) = 13.39$, $P = 0.004$; Table 2.2). VMH V1aR expression in Enriched Adult males was significantly lower than in Early Pre-Wean males and Late Pre-Wean males ($P = 0.0002$ and $P = 0.03$, respectively). VMH V1aR expression was also significantly less in Late Pre-Wean males than in Early Pre-Wean males ($P = 0.03$). No differences in VMH V1aR were found in the remaining group comparisons (***Male VMH V1aR:*** Early Pre-Wean vs Simple Adult: $P = 0.13$, Late Pre-Wean vs Simple Adult: $P = 0.47$, Enriched Adult vs Simple Adult: $P = 0.07$). In females, VMH V1aR was stable across development and showed no significant differences ($x^2(3) = 1.33$, $P = 0.72$; Table 2.4).

Lateral Septum (LS) and Central Amygdala (CeA): In both males and females, expression of LS V1aR (males: $x^2(3) = 1.87$, $P = 0.60$; females: $x^2(3) = 3.92$, $P = 0.27$; Fig 2.9, Table 2.2 and 2.4), and CeA V1aR (males: $x^2(3) = 2.35$, $P = 0.50$; females: $x^2(3) = 1.41$, $P = 0.70$; Table 2.2 and 2.4) was stable across development and showed no significant differences.

Figure 2.9. Lateral septum (LS) V1aR expression over development and due to environmental enrichment. **(A)** Autoradiogram of typical V1aR binding in the LS of an adult. Dashed line encircles the LS to indicate the borders of this region. Scale bar = 1 mm. **(B-C)** Mean (±SEM) LS V1aR (dpm/mg TE) for females and males, respectively, following Figure 2.1.



DISCUSSION

Our data strongly suggest that the developmental trajectories of OTR and V1aR throughout the forebrain of male and female prairie voles are dynamic and differ by location. Furthermore, the developmental trajectories of OTR are markedly different from those of V1aR. Whereas all OTR-expressing regions measured in males and females exhibited either no apparent change or an apparent increase over development and into adulthood, V1aR regions proved to be more dynamic, including numerous regions where expression of V1aR was highest in Early Pre-Wean and/or Late Pre-Wean (Fig 2.10). Environmental enrichment after weaning induced a remarkable sex-specific effect on V1aR development. In males only, post-weaning enrichment resulted in lower expression of V1aR in several regions; in females, this effect was only observed in the PVN (Fig 2.11, bottom). The effects of post-weaning enrichment on OTR expression were nearly identical in males and females, resulting in higher OTR expression in many regions of the brain (Fig 2.11, top). Below we expand on these general patterns across development, the effects of enrichment, and sex differences, with a limited focus on a few structures or patterns that merit commentary.

Inferring developmental trajectories of OTR and V1aR under standard laboratory housing.

In this section we focus our discussion on prairie vole patterns of receptor expression from early pre-wean to adulthood while living in standard laboratory conditions. Several forebrain regions in our study exhibited what appeared to be stable expression of OTR and V1aR over development. Indeed, in both males and females OTR did not differ for the HPC, ICp, LS, and SHi (Fig 2.10, top). Similarly, V1aR did not differ across development in the CeA, MeA, LS, or VMH in males or females (Fig 1.10, bottom). Most of these structures were also insensitive to

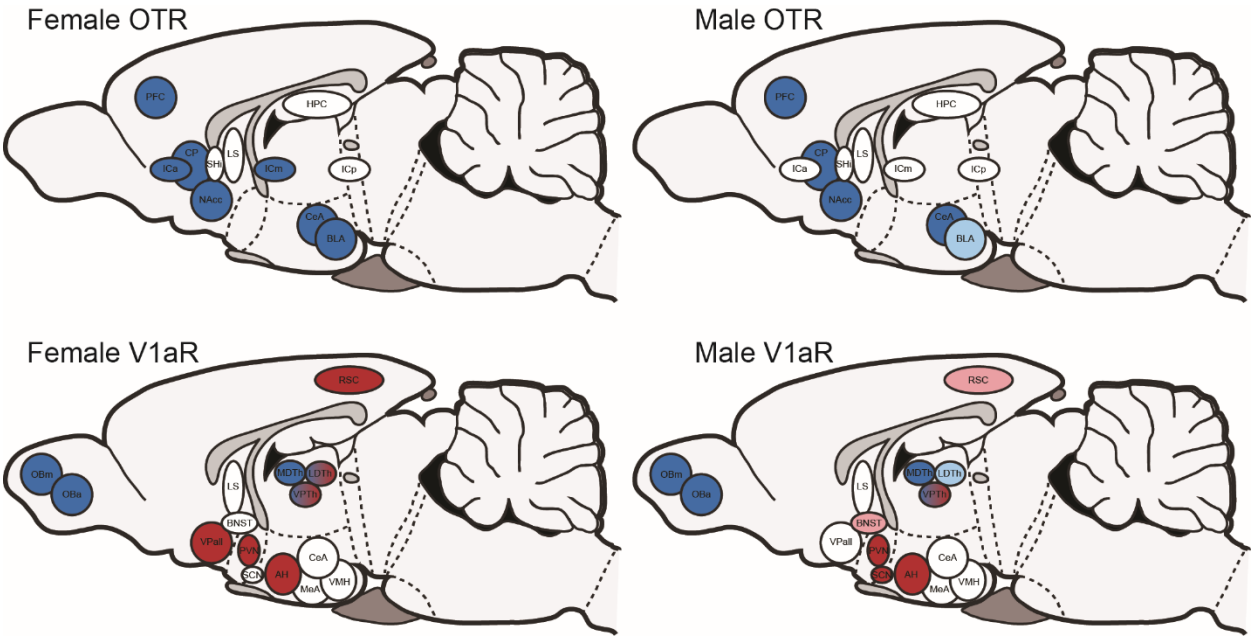
the influence of enrichment, and showed no significant differences in any comparison for either sex (OTR: ICp, HPC, LS, SHi; V1aR: CeA, LS; Fig 2.10). Presumably, receptor density reflects the likelihood that either OT or VP will bind in a particular region; the greater the receptor density, the greater the chance of binding, and thus the greater sensitivity an area is likely to have for a particular ligand. It is interesting that receptor expression appears to remain constant over development in so many brain regions. Moreover, several of these areas that showed what appears to be stable sensitivity to OT or VP are noteworthy. For example, the LS, HPC, CeA and MeA serve as central nodes within the 'social decision-making network' (O'Connell & Hofmann, 2011). The lack of differences in receptor density across development and into adulthood suggests that sensitivity to OT and VP in these structures with known roles in mediating social behavior may serve the same functions irrespective of developmental stage or age. This stability also suggests that the impact of OT and VP in these structures has a continual and central role in the development of social behavior. If changes in receptor density at different periods over development translate into differential sensitivity to nonapeptides at different ages, then the apparent stability of OTR and V1aR in these structures indicates that their functions in these areas are independent of age.

Despite this relative stability across development, many OTR and V1aR expressing areas differed as animals aged. Among these areas, OTR expression exclusively increased over development, while the patterns of change in V1aR expression were more varied. For example, regions containing V1aR were characterized by higher expression in adulthood, lower expression in adulthood, or peak expression during late pre-weaning. Specifically, we found that OTR increased in both males and females in the BLA, CeA, CP, PFC, and NAcc (Fig 2.10, top).

Females also increased OTR expression as they matured in the ICa and ICm. These differences were not seen in males. Like OTR, V1aR density also increased as animals aged in LDTh, MDTh, OBa, and OBm in both males and females (Fig 2.10, bottom). However beyond these exceptions, V1aR patterns of change differed from OTR. Specifically, V1aR expression appeared to decline over development in the AH, PVN, and RSC in both males and females, in BNST and SCN in males only, and in VPall in females only. A unique pattern of V1aR development was found in the VPTh, where V1aR expression peaked during late pre-weaning in both males and females. In the LDTh of females, we found that adults had higher V1aR expression than early pre-wean females, however the highest degree of expression occurred during late pre-weaning. In part, this suggests that V1aR in the LDTh of females follows a similar pattern to V1aR in the VPTh of both sexes.

The remarkable contrast between the developmental trajectories of OTR and V1aR expression is particularly interesting when considering that OT and VP frequently have functionally opposing influences on behavior, including learning and memory (Engelmann, Wotjak, Neumann, Ludwig, & Landgraf, 1996), anxiety (Neumann & Landgraf, 2012), and aggression (Bosch, 2013). Whether (and how) these bifurcating developmental patterns might relate to, or contribute to, the general antagonistic relationship between VP and OT is unclear. On the other hand, this observation may simply be an interesting coincidence. In either case, this pattern of opposition between VP/V1aR and OT/OTR merits further investigation.

Figure 2.10. Developmental trajectories for OTR and V1aR in male and female prairie voles. Sagittal summary schematic for OTR (top) and V1aR (bottom) expressing neural structures in females (left) and males (right). Blue represents increases in receptor expression over development. Red represents decreases in receptor expression over development. Blue-red represents a peak in receptor expression (increase then decrease) over development. Light colors represent non-significant trends. White indicates receptor density was stable over development. Abbreviations as defined in text.



Influences of post-weaning environmental enrichment on OTR and V1aR.

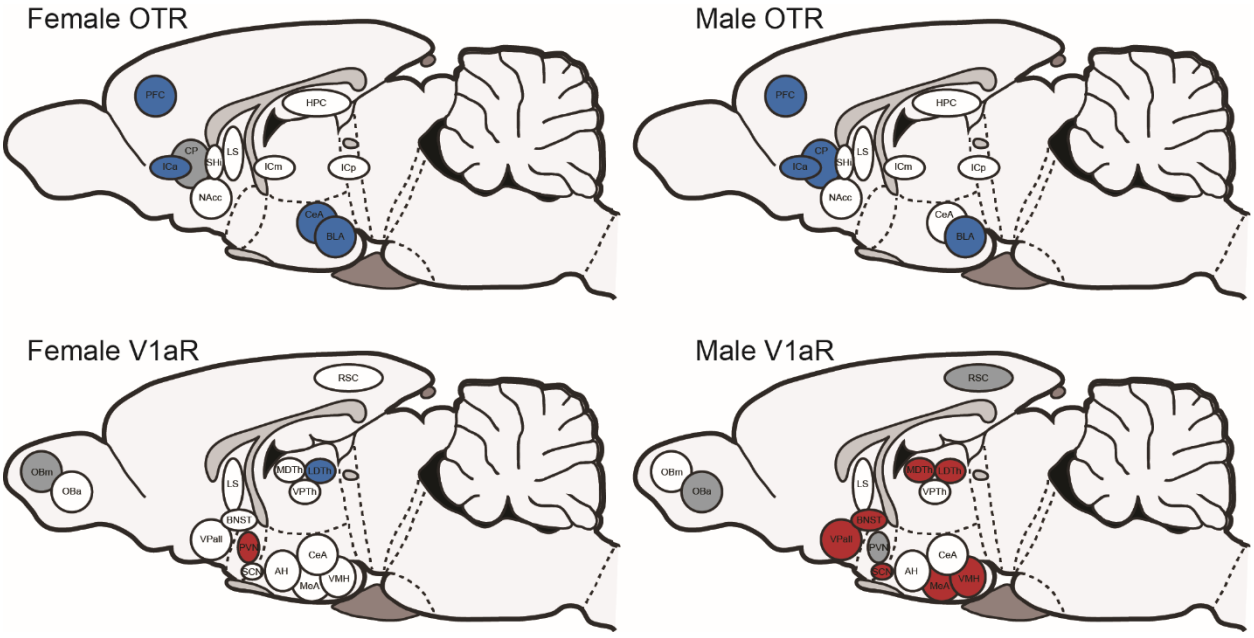
Living in a socially and spatially complex, or 'enriched', environment resulted in a variety of OTR and V1aR differences in the forebrain. That said, roughly half of the structures we investigated showed no effect of enrichment in both males and females (OTR: HPC, ICm, ICp, LS, NAcc, and SHi; V1aR: AH, CeA, LS, OBa, OBm, RSC, VPTh; Fig 2.11). In the strictest sense only four structures in males, and one structure in females showed a difference between adults living in 'Simple' and 'Enriched' conditions. These included OTR in male BLA and ICa (both of which showed more OTR expression in the enriched condition; Table 2.1), and V1aR in

male SCN and VPall (both of which showed less V1aR in the enriched condition; Fig 2.5C, Table 2.2). Only adult females in the 'Enriched' living condition showed more V1aR expression in the LDTh than in the 'Simple' living condition (Fig 2.6B, Table 2.4). However, several other areas of the forebrain appear to have been impacted by enrichment when placed in the context of development. Recall, we considered enrichment to have impacted nonapeptide receptor neural phenotype either when Enriched Adult animals were different from Late Pre-Wean animals, but Simple Adult animals were not, or when Simple and Enriched Adult values differed. Defining an effect of enrichment in these two ways captures two motifs: 1) when development has placed an animal on a particular trajectory (i.e., increasing or decreasing expression in a structure), enrichment enhances that trajectory even further (for example see OTR in the PFC of both males and females; Fig 2.1, Table 2.1 and 2.3) when a structure is apparently unaffected by development into adulthood but enrichment appears to induce a change (for example see OTR in the ICa of males; Table 2.1). Following these guidelines, our results showed that enrichment bolstered OTR expression in both males and females in the PFC, ICa, and BLA, the CP in just males, and in the CeA in just females (Fig 2.11, top). On the other hand, enrichment had a striking sex-specific effect on V1aR expression. For females, enrichment increased V1aR expression only in the LDTh, and decreased V1aR expression only in the PVN (Fig 2.11). On the other hand, enrichment decreased V1aR expression in the VPall, BNST, SCN, LDTh, MDTh, MeA, and VMH in males (Fig 2.11). Like the aforementioned patterns described over development, male OTR increased in expression while male V1aR decreased in expression when differences were found. In females, however, enrichment generally increased OTR and V1aR expression, with one exception (PVN V1aR). Five structures across males and females and receptor type demonstrated a non-significant trend for a difference between Late-Pre-Wean and

Simple Adult animals in the context of no significant difference between Simple Adult and Enriched Adult animals (see above). Based on our criteria, we did not interpret these structures to have demonstrated an effect of enrichment (see Fig 2.7c for example). These structures are marked with dark grey in Figure 2.11.

Taken together, our results support the idea that post-wean developmental environmental complexity can induce an up- or down-regulation of nonapeptide receptor expression in structures of the forebrain. In light of the known influences that nonapeptide systems have on specific forebrain regions to influence prairie vole social behavior, the alteration or exaggeration of nonapeptide expression by post-wean living conditions may have far-reaching consequences on adult behavior.

Figure 2.11. Influence of environmental enrichment on OTR and V1aR in male and female prairie voles. Sagittal summary schematic for OTR (top) and V1aR (bottom) expressing neural structures in females (left) and males (right). Blue represents increases in receptor expression resulting from enrichment. Red represents decreases in receptor expression resulting from enrichment. Dark grey represents non-significant trends suggesting no effect of enrichment. White indicates receptor density was not affected by enrichment. Abbreviations as defined in text.



Comparative perspectives of OTR and V1aR development within and between species

Our study is the first relatively exhaustive characterization of OTR and V1aR in prairie voles over development, and the only study that has also assessed the impact of post-wean enrichment on these systems. However, some earlier work investigated region-specific patterns of change over a different age range in a few structures and focused on species differences between prairie voles and their congener the montane vole (*M. montanus*) (Wang & Young, 1997; Wang et al., 1997). On balance, our results are consistent with these reports. For example, Wang et al. (1997) demonstrated that V1aR expression is reduced in the VPall and is static in the LS of male prairie voles as they age. Although Wang et al. (1997) and our study each found no evidence of receptor changes over prairie vole development in the LS, Wang and Young (1997) found increased expression of both OTR and V1aR in the LS of female prairie voles. It is difficult to reconcile the different outcomes in the LS between these studies. Methodological differences (like focus on different ages, the use of different radioligands, or possibly even neurological differences between sub-populations of voles) might explain some of these apparent idiosyncratic incongruences. Such differences do, however, raise the issue that there is a tremendous amount of individual variation in OTR and V1aR expression in prairie voles (Ophir, Campbell, et al., 2008; Ophir, Wolff, et al., 2008; S. M. Phelps & Young, 2003), and this is likely even more noticeable over the course of development. It is worth noting that pair-wise comparisons (i.e., Dunn test analysis) for LS OTR in our study suggests similar findings to those from Wang and Young (1997), however this post hoc test was not justified because overall statistical differences were not significant under the Kruskal-Wallis test.

Compared to voles, the ontogeny of OT and VP systems in rats has been well studied (Lukas, Bredewold, Neumann, & Veenema, 2010; Shapiro & Insel, 1989; Tribollet et al., 1989; Tribollet et al., 1991). Similar to our findings, trajectories of OTR and V1aR during postnatal development in rats are highly specific to region. However, the characteristic patterns of OTR and V1aR we found in prairie voles contrast with the literature in rats and other rodents. For example, unlike what we found in prairie voles, OTR expression commonly follows a trajectory of reduction in male Sprague-Dawley rats. Specifically, the NAcc, CP, and thalamic nuclei (anterior and ventricular) are all intensely labeled in young rats, but later are faint or non-labeled in adulthood (Tribollet et al., 1989). In male and female C57BL/6J mice, a similar developmental pattern of OTR expression is found in the neocortex with peak expression occurring at PND 14 (Hammock & Levitt, 2013). In prairie voles, none of the seven OTR forebrain regions that differed as a function of development exhibited a reduction in receptor expression. Taken together, OTR is relatively dynamic in rodent forebrain structures over development, and structures that change tend to follow the same trajectory within a given species. However, patterns of increases or decreases in OTR expression over development and into adulthood appear to be species specific.

In contrast to OTR in other rodent species, rat V1aR expression in most forebrain regions either increases with age or remains stable (Lukas et al., 2010; Snijdewint, Vanleeuwen, & Boer, 1989; Tribollet et al., 1991). For example, V1aR in the SCN (Tribollet et al., 1991), LS, and piriform cortex (Lukas et al., 2010) expresses more in adulthood than during pre-weaning or juvenile development. Our results showed that the olfactory bulb regions (OBm and OBa), and the laterodorsal and mediodorsal thalamus (LDTh and MDTh) were the only V1aR expressing

regions characterized by an increase with age. On the other hand, most structures (PVN, AH, VPall, and RSC) decreased V1aR expression over development. Interestingly, V1aR expression in the rat posterior cingulate cortex demonstrates a prominent peak of expression of V1aR around PND 10-13, followed by absence of binding after PND 19 (Tribollet et al., 1989). The posterior cingulate cortex is immediately anterior to the RSC, and the distinction between the two areas is poorly disambiguated structurally or functionally (Jones, Groenewegen, & Witter, 2005). The relationship between rat posterior cingulate V1aR and prairie vole RSC V1aR is unclear, but this seems to be consistent with our observation that compared to other rodents, prairie voles repeatedly demonstrate opposite themes in developmental patterns of OTR and V1aR expression. It is tempting to speculate whether these broadly defined, species-specific patterns of nonapeptide receptor development could account for some of the species-specific and developmentally regulated differences in social behaviors (e.g., pair bonding, or alloparental care), or mating tactics (see (Ophir, 2017) that distinguish prairie voles from rats and mice.

Despite the importance of comparing behavioral and neural phenotype across species (c.f., Kelly and Ophir 2015, Stevenson et al 2017, Taborsky et al 2015), to our knowledge, there are very little data exploring the developmental trajectory of nonapeptide phenotype outside rodents (see above). However some studies in non-human primates provide the opportunity to explore the extent to which the developmental differences between rodent species just described are reflected in other taxa. The available data in primates consist primarily of OTR and V1aR expression in adult rhesus macaques, common marmosets, and copper titi monkeys, and these studies indicate intriguing species differences (Freeman et al. 2014a; Freeman et al. 2014b; Schorscher-Petcu et al. 2009; Young et al. 1999). Specifically, compared to macaques and marmosets, the

development of OTR and V1aR in the socially monogamous copper titi monkey may follow a similar general rule that we found in the prairie vole: increasing OTR and decreasing V1aR between pre-weaning and adulthood. More developmental work in other taxa will provide an important foundation on which a broad understanding of nonapeptide development and function can be built (Kelly and Ophir 2015).

Some functional implications of the changes over development and / or due to enrichment

The behavioral and regulatory ramifications of the changes the nonapeptide system appears to undergo over development and as a result of enrichment holds great promise for future research. In particular, it is intriguing to consider how modification of OTR and V1aR expression throughout the social decision-making network (O'Connell & Hofmann, 2011, 2012) could result in behavioral changes during development. Such experiences may also place animals on particular trajectories that lead to different probabilities of engaging in important fitness-enhancing behaviors such as the decision to engage in a particular mating tactic, or the decision to form pair bonds - calling cards of prairie vole natural behavior (Ophir, Wolff, et al., 2008; Zheng et al., 2013).

<u>*Social behavior and social decision-making*</u>. The social behavior network (SBN) is a network of neural structures central for the regulation and modulation of social behavior and includes the Preoptic Area, CeA, MeA, BNST, LS, AH, VMH, and the midbrain (i.e., periaqueductal grey) (Goodson, 2005; Newman, 1999). This network has been expanded into a so-called social decision-making network (SDMN), which is comprised of the SDM structures and the NAcc, VPall, CP, LS, BNST, BLA HPC, and ventral tegmental area (O'Connell & Hofmann, 2011,

2012). With the exception of the preoptic area and ventral tegmental area, all of these structures express OTR, V1aR, or both in prairie voles (Zheng et al., 2013). Note, we did not observe expression in the preoptic area and we did not characterize the ventral tegmental area. Although OTR and V1aR in some of these structures were insensitive to the influence of development or enrichment, expression within several of the SBN / SDMN structures were open to these forces, including AH, BLA, BNST, CeA, CP, MeA, NAcc, VMH, and VPall. Indeed, the ways in which nonapeptides regulate neural modulation and how this may affect social behavior in maturing animals could explain much about the extent to which animals vary in social responsiveness as they age. Increases or decreases in OTR or V1aR could indicate critical periods in development where individuals might be sensitized or deafened to the effects of nonapeptide signaling. It remains to be determined whether changes in receptor expression reflect a coming 'on-line' of the nonapeptide system or a pre-adult 'tuning' of OT/VP sensitivity at different developmental periods where animals may temporarily benefit from exaggerated OT/VP neural modulation of social behavior.

Moreover, the organizational consequences of a development-by-environment interaction could profoundly impact social responsiveness and social decision-making in adults. Indeed, mounting evidence supports the degree to which social environments impact OTR and V1aR development (Curley et al., 2011). Research in rats demonstrates that social and spatial environmental enrichment leads to increased OTR in the medial preoptic area, BNST, PVN, and the CeA (Champagne & Meaney, 2008). Maternal and paternal separation manipulations in rodents have also provided evidence for the influence of early life social environments on developmental outcomes of OTR and V1aR. In male Wistar rats, maternal separation resulted in outcomes

specific to the nonapeptide system, with OTR in numerous regions (e.g., agranular insular cortex, LS, and CP) undergoing a reduction while V1aR increased (e.g., LS, and piriform cortex). We have demonstrated that pre-weaning and post-weaning social environments can interact to uniquely shape OTR and V1aR outcomes. Specifically, male prairie voles that experience single-mother rearing followed by social isolation as a juvenile are characterized by higher OTR expression in the LS (Prounis et al., 2015). Single-mother rearing had a main effect of promoting V1aR in the RSC, whereas post-weaning isolation had a main effect of promoting OTR in the PFC, BLA, and SHi (Prounis et al., 2015). Indeed, the nature in which OTR or V1aR is influenced by early social experiences is highly specific to region, and to the specific characteristics of the environmental manipulation.

Pair bonding. Prairie voles have served as the chief model for understanding the neural mechanisms for social attachment and pair bonding (Carter, Grippo, Pournajafi-Nazarloo, Ruscio, & Porges, 2008; Gobrogge & Wang, 2015; Johnson et al., 2016; L. J. Young & Wang, 2004). The accumulation of this work has outlined a central network of OT-, VP-, and dopamine-regulated neural structures that are necessary and sufficient for the formation of partner preferences and pair bonds. These include PFC, NAcc, LS, VPall, and ventral tegmental area (L. J. Young & Wang, 2004). All but one of these pair bond-governing neural structures is included in the SDMN. The network of structures that impacts pair bonding has been extended to include other supporting mechanisms and areas (including the MeA, BLA, CP, and PVN) (Johnson et al., 2016), which are also encompassed by the SDMN. Considering that social decision-making is central to the establishment of bonds, it is not terribly surprising that mechanisms important for pair bonding are largely contained within and modulated by the SBN / SDMN network.

We hypothesize that plastic responses of nonapeptide receptor systems over development and as a result of environmental enrichment may predispose an animal to adopt unique behavioral phenotypes in adulthood. For example, both males and females demonstrated an increase in PFC and NAcc OTR over development (e.g., Fig 2.12). Enrichment continued this trend in the PFC for both males and females. OT/OTR binding is sure to impact many important functions in the PFC and NAcc. However, the critical role that OT has in these structures for pair bonding seems particularly germane considering that increased OTR expression presumably translates to increased sensitivity to (or opportunity for) OT binding. In this sense, the OT system seems to come 'on-line' as animals grow, thereby enabling (among other things) the successful establishment of bonds in adults. A pattern of higher adult expression of OTR in the PFC and NAcc in males and females may therefore prepare the prairie vole brain for partner preference formation (Keebaugh & Young, 2011). When considering the species-specific nature of this increase in OTR (e.g., see above discussion of comparative perspectives), this developmental trajectory could represent a critical mechanism that led to (or was a consequence of) the evolution of the unique pair bonding behavior in prairie voles.

Although VP/V1aR binding in the VPall is also directly implicated in modulating prairie vole pair bonding (Lim & Young, 2004), we note that the lack of change in males and the apparent reduction of VPall V1aR in females over development are interesting results that merit further investigation (Fig 2.13). This is because increased pallidal V1aR facilitates at least male bonding between mating partners (Lim & Young, 2004), and we therefore would have expected for VPall in males to increase over development. The reward-modulating NAcc-VPall circuit appears to be

involved in both mediating sexual bonding and offspring bonding (Numan & Young, 2016), but it is unclear if vasopressin in the VPall in males and females functions the same in each behavioral context. For example, pregnant females show gradual decreases in pallidal V1aR as pregnancy advances (Ophir, Sorochman, Evans, & Prounis, 2013), potentially indicating that sex-specific changes in pallidal V1aR exist and follow this pattern of muted VP sensitivity in the VPall. Furthermore, it is unclear if male and female offspring differ in their propensity to bond with parents during postnatal development. Our data suggest this could be the case. For example, whereas VPall V1aR might be important for perinatal pups to bond with parents in both sexes (i.e., in early life), maintaining relatively high VPall V1aR may enable males to continually form or reinforce bonds with parents and siblings. As adults, VPall V1aR may be more important for males (possibly for pair bonding) than for females. These predicted behavioral outcomes would account for the decrease in female VPall V1aR over development.

The reduction of V1aR in the VPall of males as a result of an enriched post-wean social environment is especially perplexing. Considering that increased VP/V1aR binding in the VPall promotes bonding, reduction of V1aR in the VPall of enriched adult males should interfere with their probability of establishing partner preferences and pair bonds. It is worth noting that the specific manipulation in the enriched context exposed males to three pairs of unrelated males and no females, potentially signaling that opportunities for bonding are low and that remaining single and mating opportunistically is the best possible avenue to maximize reproductive success. This hypothesis is consistent with the idea that non-bonded prairie voles in nature might 'choose' to remain single and mate opportunistically based on the social context (Blocker & Ophir, 2016; Ophir, 2017; Ophir, Gessel, Zheng, & Phelps, 2012; S.M. Phelps & Ophir, 2009; Solomon &

Jacquot, 2002). It would also suggest that early life social experiences impact the neural mechanisms that subserve the decision to adopt particular reproductive tactics, or at least place males on trajectories that bias them to succeed within a given reproductive tactic (Blocker & Ophir, 2016; Okhovat et al., 2015; Ophir et al., 2012; Ophir, Wolff, et al., 2008).

Socio-spatial memory: We have argued elsewhere that social and spatial memory are particularly important for prairie voles to navigate their social landscape (Okhovat et al., 2015; Ophir, 2017; Ophir et al., 2012; Ophir, Wolff, et al., 2008; S.M. Phelps & Ophir, 2009). Variation in the cognitive ecology of prairie voles has likely led to individual variation in brain-behavior phenotypes and ultimately appears to shape the reproductive tactics these animals adopt. Although the focus on nonapeptides in social behavior seems to have overshadowed their roles in memory, there is indeed a rich literature on the roles of OT/OTR and VP/V1aR in regulating various forms of memory (McEwen, 2004). Based on this foundation, (Ophir, 2017) argued that nonapeptides modulate the assessment of social and spatial information, and enable prairie voles to react to their social environment in a way that will enhance their probability of successfully reproducing.

One notable pattern of results from this study is that many OTR expressing areas of the forebrain that are important for memory (HPC, SHi, LS) were fairly stable in the degree to which they expressed receptors over development and as a result of differing socio-spatial enrichment. This was not the case for V1aR expressing 'memory' areas (RSC and LDTh) where changes occurred as a result of development and/or socio-spatial enrichment. For example, LDTh V1aR expression increased over development in males and females. However, enrichment affected LDTh V1aR in

opposite directions for males and females, with expression being bolstered in females but reduced in males. RSC V1aR expression decreased over development in both males and females. Ophir et al. (2008b) first implicated RSC V1aR density in prairie vole mating tactics, leading to the overarching hypothesis that RSC V1aR impacts males' ability to encode the spatial location of conspecifics, thus leading to variable mating tactics (so-called monogamous 'residents' and non-monogamous 'wanderers') (Ophir, Wolff, et al., 2008; S.M. Phelps & Ophir, 2009). Supporting this hypothesis, polymorphisms in the prairie vole gene encoding V1aR in the RSC are related to fidelity (Okhovat et al., 2015). This allelic variation is also sensitive to functional epigenetic modification, suggesting that RSC V1aR expression responds to the social context. Moreover, the presence or absence of fathers can impact RSC V1aR expression (Prounis et al., 2015). In the current study, the RSC is one of the few regions where V1aR decreases over development in both males and females. This provides a template from which to better understand the mechanisms by which experience can shape differences in RSC V1aR expression in adults, and result in differences in behavior.

Conclusion

The region-specific developmental trajectories of OTR and V1aR we report in prairie voles highlights the dynamic nature by which nonapeptide systems are likely to regulate social behavior across the lifetime of an animal. Species-specific developmental profiles of OTR and V1aR likely contribute to interspecific variation in social behavior found in both infancy and adulthood. Many of the forebrain regions in our analysis constitute the social behavior network, and the extension of the SBN, the social decision-making network (Goodson, 2005; O'Connell & Hofmann, 2011). Given the complex nature of OTR and V1aR in regulating social behavior

across regions of this network, one should be cautious not to over-interpret the behavioral function of an isolated pattern of receptor development within a single forebrain region with exclusion of others. Focus should be placed on the significance of developmental processes that characterize multiple regions collectively. The general tendency for OTR expression to increase and V1aR expression to decrease into adulthood may broadly address socio-behavioral differences in this species. The manner in which social environments experienced during post-wean juvenile and adolescent stages alter trajectories of OTR and V1aR expression might influence significant socio-behavioral outcomes in adulthood. In prairie voles, these may include differences in pair bonding behavior or reproductive decision-making.

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Table 2.1. Oxytocin Receptor ^{125}I Binding (Mean ± SEM dpm/mg TE) across all pre-wean ages and adulthood in male prairie voles. Grey shaded columns represent the groups that were compared in statistical analyses. Early Pre Wean (EPW) ages encompassed animals postnatal days 6, 9 and 12; Late Pre Wean (LPW) ages encompassed animals postnatal days 15, 18 and 21. Significance [*] is indicated by Greek characters (α, β, and γ) that denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups (EPW, LPW, 60S, and 60E). Groups that do not share a character were significantly different. Bolded Greek characters denote non-significant trends (see *Data Analysis* in *Materials and Methods*) between groups.

	Early Pre Wean (EPW) ages							Late Pre Wean (LPW) ages							Adult (S= simple, E= enriched)			
	6		9		12		EPW		15		18		21		LPW		60S		60E	
	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N [*]	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N [*]	Mean ±SE	N [*]	Mean ±SE	N [*]
PFC	33.5 ±7.2	8	24.2 ±5.3	5	26.6 ±2.4	6	28.9 ±3.4	19 [α]	59.9 ±5.9	4	45.9 ±5.0	6	68.6 ±17.4	6	57.9 ±7.0	16 [β]	79.4 ±31.5	5 [βγ]	113.9 ±19.2	8 [γ]
ICa	51.5 ±7.5	8	39.8 ±7.2	5	47.9 ±9.8	6	47.3 ±4.7	19 [α]	57.0 ±6.0	4	41.0 ±5.6	6	53.3 ±9.0	6	49.6 ±4.4	16 [α]	43.4 ±5.0	5 [α]	76.1 ±7.9	8 [β]
NAcc	13.2 ±2.2	8	18.7 ±6.8	5	17.5 ±3.6	6	16.0 ±2.2	19 [α]	51.8 ±14.1	4	43.2 ±9.9	6	56.6 ±13.6	6	50.4 ±6.9	16 [β]	77.9 ±33.8	5 [β]	98.3 ±20.6	8 [β]
SHi	89.0 ±20.9	8	164.2 ±36.8	5	255.4 ±84.7	6	158.2 ±32.0	19 [ns]	598.4 ±203.5	4	381.0 ±171.3	6	391.4 ±169.6	6	439.2 ±99.5	16 [ns]	206.3 ±90.4	5 [ns]	244.2 ±46.6	8 [ns]
LS	34.1 ±4.6	8	34.4 ±9.6	5	53.0 ±10.1	6	40.1 ±4.7	19 [ns]	89.0 ±22.9	4	56.8 ±18.6	6	51.0 ±11.8	6	62.7 ±10.1	16 [ns]	64.6 ±11.5	5 [ns]	42.6 ±6.3	8 [ns]
CP	11.1 ±2.3	8	11.3 ±4.8	5	15.6 ±5.8	6	12.6 ±2.3	19 [α]	33.4 ±6.2	4	20.7 ±5.4	6	22.4 ±4.4	6	24.5 ±3.1	16 [β]	24.4 ±5.1	5 [βγ]	60.6 ±17.8	8 [γ]
ICm	50.2 ±3.6	8	33.5 ±7.5	5	44.6 ±8.9	6	44.0 ±3.9	19 [ns]	35.6 ±3.5	4	43.1 ±9.1	6	37.0 ±8.4	6	38.9 ±4.5	16 [ns]	52.3 ±12.2	5 [ns]	44.9 ±8.1	8 [ns]
BLA	41.6 ±12.5	8	41.0 ±9.4	5	110.8 ±52.4	6	63.3 ±18.1	19 **[α]**	94.0 ±46.9	4	139.7 ±36.8	6	151.8 ±43.0	6	132.8 ±23.4	16 [β]	88.7 ±13.6	5 **[α**β]	202.3 ±34.5	8 [γ]
CeA	41.5 ±7.7	8	65.4 ±13.4	5	117.4 ±57.0	6	71.8 ±19.1	19 [α]	146.5 ±46.7	4	153.7 ±51.4	6	134.9 ±25.6	6	144.8 ±22.9	16 [β]	103.2 ±19.5	5 [β]	197.6 ±45.0	8 [β]
HPC	75.4 ±9.7	8	119.6 ±37.4	5	136.8 ±42.7	6	106.4 ±17.3	19 [ns]	152.8 ±44.6	4	124.7 ±53.1	6	102.1 ±41.3	6	123.3 ±26.3	16 [ns]	59.4 ±17.8	5 [ns]	72.9 ±12.1	8 [ns]
ICp	45.6 ±5.5	8	24.1 ±5.7	5	25.6 ±5.6	6	33.6 ±3.9	19 [ns]	32.0 ±11.6	4	24.8 ±4.0	6	29.2 ±4.4	6	28.2 ±3.4	16 [ns]	31.1 ±7.3	5 [ns]	41.7 ±7.5	8 [ns]

Table 2.2. Vasopressin Receptor 1a ^{125}I Binding (Mean ± SEM dpm/mg TE) across all pre-wean ages and adulthood in male prairie voles. Grey shaded columns represent the groups that were compared in statistical analyses. Early Pre Wean (EPW) ages encompassed animals postnatal days 6, 9 and 12; Late Pre Wean (LPW) ages encompassed animals postnatal days 15, 18 and 21. Significance [*] is indicated by Greek characters (α, β, and γ) that denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups (EPW, LPW, 60S, and 60E). Groups that do not share a character were significantly different. Bolded Greek characters denote non-significant trends (see *Data Analysis* in *Materials and Methods*) between groups.

	Early Pre Wean (EPW) ages							Late Pre Wean (LPW) ages							Adult (S= simple, E= enriched)			
	6		9		12		EPW		15		18		21		LPW		60S		60E	
	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N [*]	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N [*]	Mean ±SE	N [*]	Mean ±SE	N [*]
OBm	30.2 ±7.4	8	31.5 ±13.0	5	59.5 ±18.2	5	38.7 ±7.3	18 [α]	78.9 ±11.8	5	87.5 ±11.7	5	96.1 ±20.8	6	88.0 ±9.0	16 [β]	177.0 ±45.0	4 [γ]	199.3 ±41.9	8 [γ]
OBa	268.0 ±34.8	8	234.6 ±37.2	5	302.9 ±50.5	5	268.4 ±22.7	18 [α]	480.0 ±52.1	5	435.3 ±35.0	5	498.2 ±40.1	6	472.9 ±24.0	16 **[β]**	611.4 ±52.4	4 **[βγ]**	551.6 ±99.0	8 [γ]
VPall	550.4 ±22.5	8	675.4 ±88.3	5	489.5 ±13.2	6	564.0 ±28.9	19 **[α]**	572.7 ±74.4	5	432.3 ±47.6	5	454.7 ±26.9	6	484.6 ±31.3	16 **[α]**	565.3 ±76.3	5 [α]	373.4 ±34.7	8 [β]
LS	216.6 ±20.7	8	312.2 ±45.9	5	225.8 ±29.6	6	244.7 ±19.0	19 [ns]	276.4 ±37.3	5	207.1 ±18.2	5	223.4 ±32.5	6	234.9 ±18.2	16 [ns]	242.2 ±31.0	5 [ns]	198.8 ±29.0	8 [ns]
BNST	208.4 ±14.1	8	199.4 ±10.5	5	201.7 ±12.4	6	203.9 ±7.3	19 **[α]**	258.0 ±39.8	5	165.4 ±10.0	5	150.2 ±15.7	6	188.6 ±17.9	16 [β]	165.8 ±25.3	5 **[αβγ]**	125.4 ±14.4	8 [γ]
PVN	134.6 ±17.7	8	114.4 ±30.2	5	107.9 ±27.8	6	120.8 ±13.4	19 [α]	124.4 ±24.4	5	55.2 ±21.0	5	90.5 ±23.4	6	90.1 ±14.3	16 **[β]**	42.5 ±8.4	5 **[βγ]**	19.1 ±7.2	8 [γ]
SCN	118.6 ±12.5	8	94.3 ±28.4	5	101.8 ±17.7	6	106.9 ±10.3	19 [α]	82.7 ±23.3	5	48.4 ±14.9	5	85.9 ±13.5	6	73.1 ±10.2	16 [β]	61.6 ±10.4	5 [β]	9.0 ±12.3	8 [γ]
AH	376.0 ±28.9	8	276.4 ±35.7	5	326.5 ±47.7	6	334.1 ±22.4	19 [α]	390.3 ±48.9	5	276.6 ±19.4	5	248.3 ±19.6	6	301.5 ±22.9	16 [α]	170.0 ±26.5	5 [β]	167.3 ±16.2	8 [β]
LDTh	124.5 ±45.6	8	547.0 ±102.2	5	603.6 ±87.8	6	387.0 ±66.6	19 **[α]**	827.5 ±50.3	4	634.2 ±64.0	5	768.3 ±80.7	6	739.4 ±43.6	15 [β]	636.9 ±103.1	4 **[α**β]	470.0 ±23.2	8 [α]
MDTh	53.4 ±15.0	8	68.3 ±10.6	5	263.2 ±58.2	6	123.6 ±29.0	19 [α]	451.2 ±82.0	4	346.3 ±57.0	5	359.5 ±52.7	6	379.6 ±35.0	15 [γ]	363.3 ±144.5	4 [βγ]	173.9 ±37.4	8 [αβ]
VPTh	130.2 ±38.7	8	276.2 ±75.9	5	383.8 ±24.7	6	248.7 ±36.0	19 [α]	448.3 ±101.8	4	312.4 ±23.8	5	370.4 ±45.1	6	371.9 ±33.6	15 [γ]	223.3 ±97.1	4 [αβ]	146.5 ±23.3	8 [β]
RSC	463.0 ±69.5	8	202.4 ±25.0	5	289.2 ±66.9	6	339.5 ±43.5	19 [α]	501.2 ±108.8	4	366.0 ±40.9	5	357.2 ±56.3	6	398.6 ±39.5	15 **[α]**	244.4 ±119.9	4 **[α**β]	135.7 ±23.7	8 [β]
CeA	245.0 ±32.6	8	228.0 ±35.3	5	149.9 ±25.8	6	210.5 ±20.0	19 [ns]	175.1 ±53.3	4	206.9 ±29.2	5	170.3 ±39.1	6	183.8 ±21.9	15 [ns]	211.4 ±42.9	4 [ns]	158.8 ±19.1	8 [ns]
MeA	192.5 ±21.5	8	183.7 ±30.0	5	219.4 ±30.0	6	198.7 ±14.8	19 [α]	201.3 ±17.3	4	159.8 ±29.9	5	146.7 ±28.8	6	165.6 ±16.0	15 [α]	159.2 ±31.7	4 [αβ]	111.4 ±13.9	8 [β]
VMH	281.5 ±36.7	8	266.3 ±46.9	5	245.9 ±33.0	6	266.3 ±21.4	19 [α]	260.6 ±36.0	4	187.4 ±34.3	5	164.8 ±34.6	6	197.9 ±21.6	15 [β]	194.6 ±55.9	4 [αβγ]	129.0 ±14.9	8 [γ]

Table 2.3. Oxytocin Receptor ^{125}I Binding (Mean ± SEM dpm/mg TE) across all pre-wean ages and adulthood in female prairie voles. Grey shaded columns represent the groups that were compared in statistical analyses. Early Pre Wean (EPW) ages encompassed animals postnatal days 6, 9 and 12; Late Pre Wean (LPW) ages encompassed animals postnatal days 15, 18 and 21. Significance [*] is indicated by Greek characters (α, β, and γ) that denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups (EPW, LPW, 60S, and 60E). Groups that do not share a character were significantly different. Bolded Greek characters denote non-significant trends (see *Data Analysis* in *Materials and Methods*) between groups.

	Early Pre Wean (EPW) ages								Late Pre Wean (LPW) ages								Adult (S= simple, E= enriched)			
	6		9		12		EPW		15		18		21		LPW		60S		60E	
	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N [*]	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N [*]	Mean ±SE	N [*]	Mean ±SE	N [*]
PFC	73.8 ±7.3	9	66.6 ±11.7	5	85.6 ±25.8	5	75.0 ±7.8	19 [α]	169.1 ±21.2	5	243.8 ±41.6	5	232.2 ±50.3	5	215.0 ±22.9	15 [β]	276.5 ±28.1	6 [βγ]	349.8 ±41.9	8 [γ]
ICa	73.9 ±16.3	9	41.2 ±14.1	5	85.8 ±23.8	5	68.5 ±10.8	19 [α]	84.4 ±22.9	5	149.2 ±25.6	5	92.2 ±18.0	5	108.6 ±14.3	15 [β]	112.1 ±21.4	6 [βγ]	181.3 ±30.1	8 [γ]
NAcc	63.0 ±23.4	9	54.1 ±14.0	5	145.5 ±27.8	5	82.4 ±15.9	19 [α]	132.3 ±22.6	5	134.1 ±24.6	5	171.7 ±59.0	5	146.0 ±21.5	15 [β]	203.1 ±48.9	6 [β]	193.6 ±38.4	8 [β]
SHi	219.9 ±50.0	9	407.4 ±121.1	5	503.3 ±156.2	5	343.8 ±60.4	19 [ns]	466.3 ±49.6	5	621.8 ±62.4	5	466.5 ±155.0	5	518.2 ±57.2	15 [ns]	497.7 ±103.1	6 [ns]	493.1 ±108.2	8 [ns]
LS	121.8 ±24.0	9	166.4 ±36.9	5	269.0 ±31.9	5	172.2 ±21.6	19 [ns]	237.9 ±45.7	5	250.7 ±39.1	5	234.6 ±27.9	5	241.1 ±20.6	15 [ns]	243.6 ±51.7	6 [ns]	234.8 ±44.2	8 [ns]
CP	81.9 ±12.7	9	71.7 ±22.1	5	104.4 ±27.2	5	85.1 ±10.7	19 **[α]**	97.3 ±11.1	5	137.7 ±11.9	5	106.1 ±20.2	5	113.7 ±9.3	15 **[αβ]**	190.9 ±45.0	6 **[β**γ]	180.8 ±23.3	8 [γ]
ICm	118.4 ±11.5	9	80.0 ±21.0	5	120.8 ±41.9	5	108.9 ±13.2	19 [α]	132.2 ±27.5	5	169.3 ±24.4	5	123.3 ±50.8	5	141.6 ±20.1	15 [β]	200.7 ±21.0	6 [γ]	191.7 ±14.8	8 [γ]
BLA	225.3 ±37.1	9	202.9 ±31.3	5	191.0 ±41.2	5	210.4 ±21.4	19 [α]	310.8 ±54.7	5	280.7 ±40.7	5	254.0 ±74.5	5	281.8 ±31.8	15 [β]	340.6 ±59.9	6 [βγ]	390.1 ±41.9	8 [γ]
CeA	247.1 ±27.1	9	220.7 ±45.4	5	268.4 ±69.0	5	245.8 ±23.9	19 [α]	360.3 ±81.1	5	313.2 ±45.4	5	313.1 ±60.2	5	328.9 ±34.7	15 [β]	411.7 ±73.1	6 [βγ]	511.7 ±57.5	8 [γ]
HPC	193.9 ±39.0	9	340.7 ±73.0	5	300.3 ±87.0	5	260.5 ±36.2	19 [ns]	447.2 ±98.7	5	312.4 ±48.6	5	301.8 ±84.4	5	353.8 ±46.3	15 [ns]	341.8 ±36.5	6 [ns]	272.8 ±30.8	8 [ns]
ICp	159.5 ±23.7	9	252.5 ±52.9	5	219.8 ±53.8	5	199.8 ±23.2	19 [ns]	313.0 ±74.0	5	240.6 ±45.5	5	254.8 ±55.4	5	269.5 ±32.9	15 [ns]	286.1 ±28.3	6 [ns]	261.4 ±23.4	8 [ns]

Table 2.4. Vasopressin Receptor 1a ^{125}I Binding (Mean ± SEM dpm/mg TE) across all pre-wean ages and adulthood in female prairie voles. Grey shaded columns represent the groups that were compared in statistical analyses. Early Pre Wean (EPW) ages encompassed animals postnatal days 6, 9 and 12; Late Pre Wean (LPW) ages encompassed animals postnatal days 15, 18 and 21. Significance [*] is indicated by Greek characters (α, β, and γ) that denote significant *post hoc* comparisons ($P \leq 0.05$), with shared characters indicating statistical similarity between groups (EPW, LPW, 60S, and 60E). Groups that do not share a character were significantly different. Bolded Greek characters denote non-significant trends (see *Data Analysis* in *Materials and Methods*) between groups.

	Early Pre Wean (EPW) ages								Late Pre Wean (LPW) ages								Adult (S= simple, E= enriched)			
	6		9		12		EPW		15		18		21		LPW		60S		60E	
	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N [*]	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N	Mean ±SE	N [*]	Mean ±SE	N [*]	Mean ±SE	N [*]
OBm	478.4 ±116.4	7	520.4 ±135.1	5	834.3 ±189.0	5	595.5 ±87.0	17 [α]	717.3 ±115.7	5	1258.0 ±257.7	5	1224.8 ±270.2	5	1066.7 ±137.6	15 **[β]**	1583.7 ±227.5	9 **[β**γ]	1905.5 ±313.9	8 [γ]
OBa	2626.0 ±380.7	7	2982.1 ±427.3	5	3797.7 ±424.5	5	3075.4 ±253.0	17 [α]	4358.5 ±255.5	5	5756.9 ±285.2	5	4994.9 ±468.9	5	5036.8 ±241.3	15 [β]	5051.2 ±217.6	9 [β]	5632.3 ±396.4	8 [β]
VPall	5882.0 ±401.2	7	5767.3 255.6	5	5183.0 ±211.4	5	5642.7 ±196.4	17 **[α]**	5320.1 ±339.0	5	5198.0 ±435.7	5	5177.4 ±243.4	5	5231.8 ±187.0	15 **[α**β]	4757.6 ±205.4	9 [β]	4735.8 ±262.1	8 [β]
LS	1732.7 ±204.0	7	2217.1 ±494.3	5	1910.0 ±111.2	5	1927.3 ±166.8	17 [ns]	2291.8 ±277.8	5	2503.8 ±339.8	5	2390.8 ±361.3	5	2395.5 ±177.0	15 [ns]	2381.8 ±383.8	9 [ns]	2475.7 ±374.2	8 [ns]
BNST	2730.3 ±214.6	7	2409.3 ±222.8	5	2885.8 ±327.6	5	2681.6 ±144.4	17 [ns]	2790.0 ±343.8	5	2556.8 ±406.5	5	2843.0 ±177.2	5	2730.0 ±176.3	15 [ns]	2344.3 ±135.9	9 [ns]	2484.7 ±180.7	8 [ns]
PVN	1528.9 ±137.8	7	2016.8 ±100.9	5	1496.9 ±176.5	5	1663.0 ±96.1	17 [α]	1254.3 ±190.6	5	744.7 ±172.2	5	1491.7 ±213.1	5	1163.6 ±132.5	15 [β]	863.5 ±228.7	9 [βγ]	705.6 ±79.5	8 [γ]
SCN	1582.7 ±298.7	7	1711.7 ±183.9	5	1230.4 ±207.7	5	1517.0 ±147.5	17 [ns]	1167.5 ±267.1	5	1042.1 ±238.8	5	1616.4 ±224.9	5	1275.3 ±146.2	15 [ns]	1058.3 ±170.2	9 [ns]	1219.8 ±174.7	8 [ns]
AH	3508.8 ±314.4	7	4142.0 ±375.6	5	3527.3 ±536.8	5	3700.4 ±227.8	17 [α]	2956.5 ±358.9	5	2866.5 ±436.4	5	3216.6 ±174.9	5	3013.2 ±186.8	15 [β]	2301.0 ±249.6	9 [γ]	2401.9 ±192.0	8 [γ]
LDTh	1188.6 ±476.2	7	4468.0 ±556.5	5	5093.3 ±427.2	5	3301.5 ±519.7	17 [α]	5375.5 ±733.7	5	5732.5 ±215.0	5	5120.8 ±1197.7	5	5409.6 ±443.6	15 [β]	4409.0 ±366.6	8 [γ]	5572.3 ±489.0	8 [β]
MDTh	-92.0 ±119.5	7	959.0 ±669.3	5	2481.0 ±647.6	5	973.9 ±370.1	17 [α]	2286.5 ±516.2	5	3207.4 ±672.5	5	3875.1 ±955.3	5	3123.0 ±430.9	15 [β]	2292.6 ±540.1	8 [β]	3156.6 ±540.0	8 [β]
VPTh	15.3 ±119.5	7	1975.5 ±669.3	5	1983.0 ±647.6	5	1170.6 ±352.2	17 [α]	2979.7 ±516.2	5	3148.8 ±672.5	5	3827.8 ±955.3	5	3318.8 ±451.6	15 [β]	1276.8 ±540.1	8 [α]	1992.4 ±540.0	8 [α]
RSC	2363.6 ±414.4	7	3624.5 ±582.9	5	4233.6 ±185.9	5	3284.5 ±307.5	17 [α]	3826.2 ±595.3	5	3223.5 ±694.9	5	3321.9 ±578.3	5	3457.2 ±341.4	15 **[α]**	1326.5 ±541.1	9 [β]	2428.2 ±428.2	8 **[α**β]
CeA	3449.6 ±295.8	7	3168.1 ±606.9	5	2712.3 ±666.5	5	3150.0 ±281.2	17 [ns]	1935.7 ±435.7	5	2865.0 ±542.7	5	3376.8 ±440.4	5	2725.8 ±300.0	15 [ns]	3174.7 ±347.1	9 [ns]	3181.1 ±408.9	8 [ns]
MeA	2069.0 ±283.0	7	2639.7 ±347.7	5	2318.6 ±334.4	5	2310.3 ±181.6	17 [ns]	2040.2 ±354.0	5	2570.0 ±156.8	5	3275.5 ±596.1	5	2628.5 ±257.7	15 [ns]	2430.9 ±196.1	9 [ns]	2976.2 ±344.8	8 [ns]
VMH	3427.8 ±249.2	7	4154.9 ±401.3	5	3198.8 ±448.4	5	3574.3 ±213.4	17 [ns]	2875.4 ±517.4	5	3526.0 ±466.3	5	3727.8 ±314.8	5	3376.4 ±255.1	15 [ns]	3123.6 ±215.6	9 [ns]	3262.9 ±236.4	8 [ns]

CHAPTER 3 – Perinatal social environments interact to shape cognitive behavior and neural phenotype in prairie voles

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ABSTRACT

Social environments experienced at different developmental stages profoundly shape adult behavioral and neural phenotypes, and may have important interactive effects. We asked if social experience before and after weaning influenced adult social cognition in male prairie voles. Animals were raised either with or without fathers and then either housed singly or in sibling pairs. Males that were socially deprived before (fatherless) and after (singly housed) weaning did not demonstrate social recognition or dissociate spatial from social information. We also examined oxytocin and vasopressin receptors (OTR and V1aR) in areas of the forebrain associated with social behavior and memory. Pre- and post-wean experience differentially altered receptor expression in several structures. Of note, OTR in the lateral septum - an area in which oxytocin inhibits social recognition - was greatest in animals that did not clearly demonstrate social recognition. The combination of absentee fathers on V1aR in the retrosplenial cortex and single housing on OTR in the septohippocampal nucleus produced a unique phenotype previously found to be associated with poor reproductive success in nature. We demonstrate that interactive effects of early-life experiences throughout development have tremendous influence over brain-behavior phenotype and can buffer potentially negative outcomes due to social deprivation.

INTRODUCTION

The quality and composition of the postnatal social environment can profoundly affect brain organization, and this can have cascading effects that alter developmental trajectories into adulthood (Kundakovic & Champagne, 2015). Indeed, most species face highly dynamic and variable early life social experiences, and the extent to which social environments can shape both the brain and behavior has remained an area of potent interest. Despite the clear importance that early life experiences have on shaping phenotypes, most studies have focused on single developmental periods and have overlooked a crucial element: social experiences at different developmental periods might interact. Indeed ignoring that development is unidirectional in time and builds on previous experience also ignores that the adult phenotype is the outcome of complex phenotypic sculpting by many early life experiences. Examining the interactions of varied environments at distinct developmental stages is necessary if we are to meet the goal of providing a more thorough understanding of how adult behavioral and neural phenotypes emerge.

Pre-weaning stages of development in rodents are incredibly influential. Most work on the influences of early life social environments has focused on the impact of pre-weaning mother-infant interactions (Champagne, 2013; Lukas, Bredewold, Landgraf, Neumann, & Veenema, 2011), largely because most mammals only engage in uni-parental care (Kleiman, 1977). It is, however, also particularly important to understand how father-infant interactions influence offspring development in bi-parental species. Relatively few non-human studies have investigated the effects of paternal care and the importance of fathers at the nest (Braun & Champagne, 2014). Such studies have identified several socio-behavioral deficits associated with

paternal absence. For example, the absence of fathers in the natal nest of bi-parental rodents adversely influences maturation, partner-preference formation, alloparental behavior, and social recognition in adulthood (Ahern & Young, 2009; Cao et al., 2014; Z. Wang & Novak, 1994; Z. X. Wang & Novak, 1992). However, in order to fully understand the influence of early life experiences on development, we must consider the impact of social environments beyond the natal nest. Adolescence and early adulthood are also periods of life known to assert a profound influence over developmental trajectories. For example, comparisons of rodents living in isolated vs. group housing conditions post-weaning suggest that isolated animals develop high anxiety-like phenotypes and stress-induced behavioral and neuroendocrine changes (Grippo et al., 2007; Lukkes, Watt, Lowry, & Forster, 2009; Pan, Liu, Young, Zhang, & Wang, 2009), indicating that the social environment continues to impact development.

Post-weaning social environments could potentially impact development in a manner that is distinct from pre-weaning social environments. Furthermore, because these important developmental stages are separated in time, they may interact to shape neural phenotype and social behavior in complex ways. For example, environmental enrichment in later life modifies differences in neurodevelopment and anxiety-like behavior that emerge from having high or low licking and grooming mothers (Champagne & Meaney, 2007). Post-wean environmental enrichment also reverses the influence of impoverished mother-pup interactions on hippocampal physiology and spatial memory (Bredy, Humpartzoomian, Cain, & Meaney, 2003; Bredy, Zhang, Grant, Diorio, & Meaney, 2004). These studies underscore the need to consider how brain and behavior can be affected by interactions between social environments in early and late development. Doing so will enable a deeper understanding of social development in ways that

focusing on social experiences at single developmental periods does not capture.

Prairie voles (*Microtus ochrogaster*) provide an excellent opportunity to investigate how early social experiences at different life stages interact to shape complex brain-behavior phenotypes. Prairie voles are most known for their tendencies to form long-term pairbonds (Carter, 1998; Young & Wang, 2004) and their socially monogamous mating system is closely associated with bi-parental care. While most breeding units in nature consist of a heterosexual pair (referred to as 'residents') and their offspring, some reproductively active animals are single due to a member of the pair defecting ('divorce'), predation, or what appears to be an active choice to forgo pairbonding (Getz, McGuire, Pizzuto, Hofmann, & Frase, 1993; McGuire, Getz, Bemis, & Oli, 2013; Ophir, Phelps, Sorin, & Wolff, 2008). Furthermore, 'wandering' individuals can be male or female (Getz et al., 1993; McGuire et al., 2013; Ophir, Phelps, et al., 2008; Zheng, Larsson, Phelps, & Ophir, 2013). These differences in mating tactics result in natural variation in the postnatal social environment; both parents will raise some offspring, and just mothers will raise others. Moreover, as pups mature into sub-adults, some continue to reside at the nest, while others leave the nest to join the reproductive population. Thus, sometime after weaning, some sub-adult animals will live in social groups, while others will live singly.

The neurobiology that modulates prairie vole pairbonds is also well understood. Much of this work has focused on the nonapeptides oxytocin (OT) and vasopressin (VP). For example, region specific manipulations of OT, VP, or their receptors (OTR and V1aR) in the lateral septum (LS, (Liu, Curtis, & Wang, 2001)), ventral pallidum (VPall, (Lim & Young, 2004)), nucleus accumbens (NAcc, (Aragona, Liu, Curtis, Stephan, & Wang, 2003; Ross et al., 2009)), and prefrontal cortex (PFC, (Young, Lim, Gingrich, & Insel, 2001)) can enhance or eliminate the

propensity to bond, which has led to their inclusion in a 'pairbonding neural circuit' (Young & Wang, 2004). Not surprisingly, most of these structures are deeply associated with the 'social behavior network' (Goodson, 2005; Newman, 1999), a set of nuclei that are frequently implicated in modulating social behaviors and in which nonapeptides assert a tremendous influence. What has been less appreciated recently, but has deep historical roots, is the influence of OT and VP in memory (Bohus, Kovacs, & Dewied, 1978; McEwen, 2004). Although much of the original work was on avoidance and appetitive learning, more recently their roles in social recognition (in the LS) and spatial memory (in the septohippocampal nucleus (SHi), hippocampus (Hi), and the retrosplenial cortex (RSC)) have been topics of interest (Ferguson, Young, & Insel, 2002; Ophir, Gessel, Zheng, & Phelps, 2012; Ophir, Wolff, & Phelps, 2008; Ophir, Zheng, Eans, & Phelps, 2009). In addition to their aforementioned influences on social behavior and memory, nonapeptide systems are open to the influences of early-life social experience in numerous species, including prairie voles (Ahern & Young, 2009; Bales, Boone, Epperson, Hoffman, & Carter, 2011; Bales & Perkeybile, 2012; Carter, 2003).

Here, we evaluate the interactive influences of pre-weaning (presence or absence of fathers) and post-weaning (group- vs. single-housed) social environments on oxytocin and vasopressin receptor expression across brain regions of the pairbonding neural circuit, social behavior network, and socio-spatial memory structures in male prairie voles. Because social and spatial memory arguably form the foundation of social cognition (knowing who and where conspecifics are in space), we focus on performance in a modified social discrimination test that places social discrimination into a spatial context to ask how early life experiences from different stages of development affect socio-spatial memory in adults.

METHODS

Animals

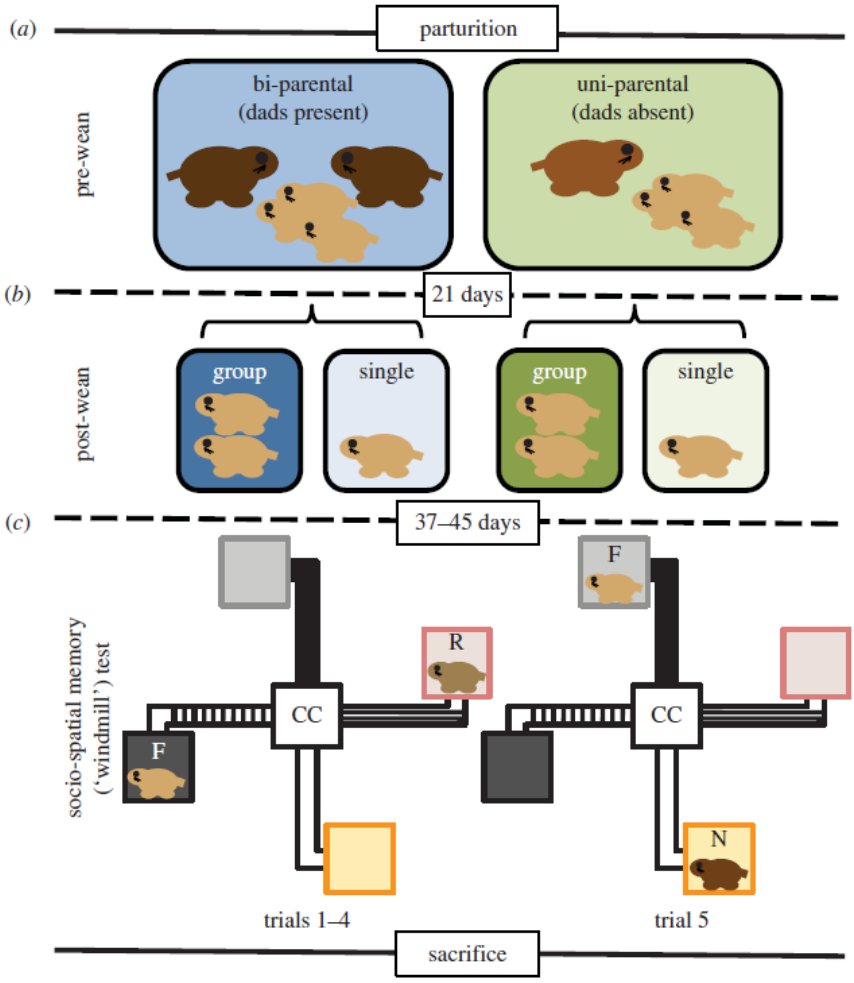
All subjects came from the first litter of breeding pairs (N = 64) created specifically for this experiment. The breeders originated from our colony of voles, which were originally trapped in Champaign County, Illinois. The animals were housed in standard polycarbonate rodent cages (29 x 18 x 13 cm) and kept on a 14:10 light:dark cycle, with lights on at 0600. They were provided Rodent Chow 5000 (Harlan Teklad, Madison, WI, U.S.A.) and water *ad libitum*. After pairing, mating behavior was monitored closely and used to estimate the expected parturition date. All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Oklahoma State University.

Early life manipulations

We created four rearing conditions (a 2 x 2 design) that contrasted pre-wean and post-wean social experience (see Fig 3.1a-b). Just before females were expected to give birth, we closely checked breeding pairs daily for newborn offspring. We recorded the number of offspring when a new litter was discovered. When pups were born, the fathers from half of the breeding pairs were removed from the cage ('dad absent'); the other half of the fathers was left undisturbed ('dad present'). The conditions were maintained until the pups were 21 days old, at which point the pups were sexed and weaned. At weaning, half of the pups from each group (dads absent or present) were assigned into post-wean groups. Animals serving in group-housed treatments were housed with a same-sex littermate; animals serving in single-housed treatments were housed alone. Post-wean housing conditions were maintained for the duration of the study. Behavioral testing began just after animals reached sub-adult age (~35 days). Our behavioral experiments

focused on males. Taken together we created four groups: dad present/group-housed (N = 15), dad present/ single-housed (N = 14), dad absent/group-housed (N = 18), or dad absent/ single-housed (N = 17).

Figure 3.1. Experimental design and timeline of early life social manipulations and the socio-spatial memory test. (A) Between parturition and weaning (21d) subjects were reared bi-parentally (blue) or uni-parentally (green). (B) Subjects were then housed with a same-sex sibling (group; dark shade) or alone (single; light shade). (C) Between 37d-45d, all subjects were tested in a socio-spatial memory test. The experimental set up for the socio-spatial memory test trials 1-4 is depicted on the left; the set up for the final test trial is depicted on the right. Hypothetical placement of the familiar stimulus animal used throughout the five trials (F), the familiar stimulus animal that was used in trials 1-4 and removed in trial 5 (R), and the novel stimulus animal presented only in trial 5 (N) is presented to illustrate the experimental procedure. Color and shades of the stimulus chambers correspond to figures below (grey: location of F in trials 1-4 [dark] and trial 5 [light]; red: location of R in trials 1-4; orange: location of N in trial 5). CC = center chamber, where the subject was placed at the beginning of each trial. Brains were extracted and processed after serving in the behavioral test.



Socio-spatial memory test

Between 37-46 days old, subject males were tested in a socio-spatial memory test (Fig 3.1c). This test functions as a modified social discrimination test (Engelmann, Wotjak, & Landgraf, 1995) that incorporates social recognition in a spatial context. Traditional social discrimination tests present a subject with two unfamiliar conspecifics over a series of presentations to give the subject the opportunity to become familiar with the two stimulus animals (a familiarization phase). Social investigation is expected to decline as a subject becomes more familiar with the stimulus conspecifics. To test recognition, one of the two familiar stimulus animals is replaced by a third novel stimulus animal, and the time the subject spends investigating the novel animal compared to the familiar animal provides a measure of social discrimination. A subject is expected to spend more time investigating the novel animal, presumably because it is gathering information about the new identity.

In our test, two stimulus subjects were placed in the same respective arms of a four-arm apparatus (Fig 3.1c, left; see below) during the familiarization phase. At the time of the test, one familiar stimulus animal was moved to a previously unoccupied arm, while the other was removed and an unfamiliar stimulus animal was placed in the other previously unoccupied arm (Fig 3.1c, right). Thus, our test was designed to assess social recognition, but in a context where the spatial associations between identity and location were broken to contrast the reliance on spatial and social information. In any given trial, all stimulus and subject animals were unrelated.

Apparatus

The acrylic apparatus resembled the arms of a windmill (Fig 3.1c) and consisted of a central chamber (22.86 x 22.86 cm) that opened into four distinctly marked hallways (45.7 x 7.6 cm). Each hallway turned a corner at the distal end (7.6 x 7.6 cm), at which point a wire mesh barrier separated the hallway from an end chamber (22.86 x 22.86 cm) in which stimulus animals could be presented. Each arm was uniquely marked (black, white, horizontal stripes, or vertical stripes) to enable local cue discrimination. The end chambers were placed around a corner so that subject animals were unable to see if they contained a stimulus animal without walking to the end of each hallway.

Procedure

To begin a trial, subjects were placed in the central chamber for 30 min. During this time, opaque acrylic squares blocked access to the hallways. Two stimulus animals were placed on opposite ends of the apparatus from each other in their own end chamber. Their placement with respect to the arm markings was random. The acrylic squares were removed, and the subject was allowed to explore the apparatus freely for 5 min. Next, the subject was returned to the central chamber and the acrylic squares were replaced to block access to the hallways for 15 minutes. This procedure was repeated five times. Before the fifth presentation period began, one stimulus animal was rotated to the previously unoccupied arm to the right to serve as a familiar stimulus animal (F). The other stimulus animal was removed (R) and replaced with a third novel stimulus animal (N) that was placed in the remaining previously unoccupied presentation chamber (see Fig 3.1c).

We used EthoVisionXT (Noldus Information Technology, Leesburg, VA) to measure the time spent in the main chamber and in each of the four arms for each trial. We subdivided each arm into the hallway and a 'social interaction zone' (SIZ; 15.2 x 7.6 cm). Each SIZ was approximately 1.5 body lengths. In trial 5, the difference between time spent in the SIZs of the arms containing stimulus animals indicated if subjects discriminated between familiar and unfamiliar conspecifics. The time spent in the SIZs of the vacant chambers (where stimulus animals were previously housed during trials 1-4) indicated a subject's ability to dissociate previously paired social and spatial information. In other words, it assessed the reliance on spatial cues and social cues.

OTR and V1aR autoradiography

After behavioral trials were complete, subjects were sacrificed and we immediately extracted brains, froze them on powdered dry ice, and stored them at -80 °C. Later, we coronally cryosectioned brains at 20 µm and mounted sections at 100 µm intervals on Superfrost Plus slides (Fisher Scientific Co., Pittsburgh, PA, USA). Each of four sets was then stored at -80 °C until they were used to visualize receptor density using autoradiography as previously described (Ophir, Sorochman, Evans, & Prounis, 2013). We used ^{125}I-labeled radioligands to visualize oxytocin receptor (ornithine vasotocin analogue ([^{125}I]-OVTA); NEX254, PerkinElmer; Waltham, MA, USA) and vasopressin receptor (vasopressin (Linear), V-1A antagonist (Phenylacetly1, O-Me-D-Tyr2, [^{125}I-Arg6]-); NEX310, PerkinElmer). We exposed radiolabelled tissue to film (GE Healthcare, Little Chalfont, UK) for 4 days. The relative density of ligand binding was assessed by inferring that receptor density relates to the optical density of exposed film and, in this way, optical density measurements serve as a proxy for receptor density. We

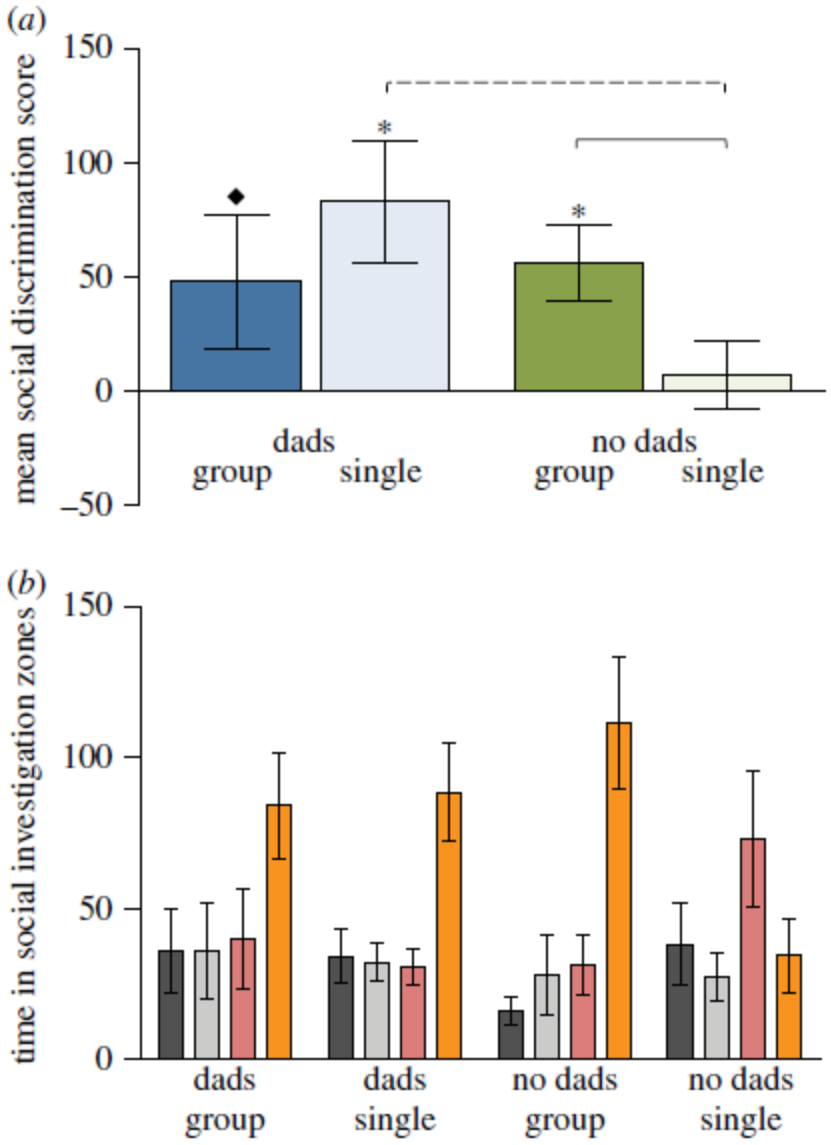
used ^{125}I-labelled radiographic standards (American Radiolabeled Chemicals, St Louis, MO, USA) to allow for conversion of optical density to receptor density. We digitized films on a Microtek ArtixScan M1 (Microtek, Santa Fe Springs, CA, USA) and measured optical densities using IMAGE-J (NIH, Bethesda, MD, USA). We calculated receptor density by first converting optical density to disintegrations per minute (dpm), adjusted for tissue equivalence (TE; for 1 mg in the rat brain), by using a log function to fit curves generated by radiographic standards. We measured optical density across brain regions within the pairbonding circuit, social behavior network, and memory circuits (OTR: prefrontal cortex [PFC], nucleus accumbens [NAcc], septo-hippocampal nucleus [SHi], lateral septum [LS], hippocampus [Hi], central amygdala [CeA], basolateral amygdala [BLA]; V1aR: ventral pallidum [VPall], lateral septum [LS], medial bed nucleus of the stria terminalis [BSTm], anterior hypothalamus [AH], retrosplenial cortex [RSC], central amygdala [CeA], medial amygdala [MeA], and ventromedial hypothalamus [VMH]). The optic density for each brain region was averaged over three measurements (once in a series of three brain sections, bilaterally). We also measured optical density of portions of film adjacent to the brain slices analyzed. The average values for each structure were converted to dpm/mg TE, and adjusted for film effects by subtracting film optical densities from binding measurements. Some tissue sections were damaged during processing. As a result, sample sizes slightly varied by structure and receptor type (see Supplemental Table S3.1).

RESULTS

Early life social environments, social discrimination and socio-spatial memory

To begin, we first asked if early life social experience affected social discrimination. Social discrimination scores were calculated by subtracting the time spent in the SIZ of the familiar stimulus animal from the time spent in the SIZ of the novel stimulus animal in trial 5. To assess the degree of social discrimination, we compared the mean social discrimination scores to zero using one-sample t-Tests (Fig 3.2a). For all statistical tests we used $\alpha = 0.05$. Social discrimination scores for dad present/single-housed and dad absent/group-housed males were significantly greater than zero ($t_{17} = 3.367$, $P = 0.004$; $t_{13} = 3.129$, $P = 0.008$, respectively), demonstrating social discrimination. Dad present/group-housed males did not significantly differ from zero, but they did show a non-significant tendency toward social discrimination ($t_{14} = 1.64$, $P = 0.12$; Fig 3.2a). Males in the dad absent/single-housed group clearly did not differ from zero ($t_{16} = 0.4729$, $P = 0.64$), indicating potential deficits in social discrimination.

Figure 3.2. (A) Mean (±SE) male social discrimination scores were calculated by taking the average of the differences in time subjects were located in the social interaction zone (SIZ) of the novel and familiar stimulus animals in trial 5 for each treatment group. Males that were reared with their fathers present are colored blue; males raised without fathers are colored green. Dark shades indicate that males were group housed post-wean; light shades indicate that males were housed alone. Star indicates the treatment group was significantly greater than zero; diamond indicates a non-significant trend. Solid ($p \leq 0.05$) and dashed ($p \leq 0.01$) lines indicate significant post hoc differences between groups. (B) Mean (±SE) time (in seconds) males spent in the SIZ of each stimulus chamber in trial 5 for males from each rearing treatment. Colors for bars correspond to Figure 2.1c: (grey: location of the familiar stimulus animal (F) in trials 1-4 [dark] and trial 5 [light]; red: location of the familiar stimulus animal that was removed for trail 5 (R); orange: location of the novel stimulus animal in trial 5 (N).



When the social discrimination scores across the four treatment groups were analyzed with a 2-way ANOVA, there was no significant main effect of pre-weaning ($F_{1, 60} = 2.41$, $P = 0.13$) or post-weaning ($F_{1, 60} = 0.10$, $P = 0.75$), but the data did show a significant interaction between the two treatments ($F_{1, 60} = 3.71$, $P = 0.05$; Fig 3.2a), where dad absent/single-housed males had significantly lower social discrimination scores than males raised with dads and housed alone (post hoc Student's *t*-Test; $t_{29} = 2.609$, $P = 0.01$) or without dads and group housed ($t_{33} = 2.183$, $P = 0.04$). However, males raised without dads and housed alone did not significantly differ from males raised with dads and housed in groups ($t_{30} = 1.288$, $P = 0.21$). Taken together, the significant interaction supports the hypothesis that the combination of pre-and post-wean socially depleted environments disrupt or delay social discrimination.

We compared the amount of time that subjects from each group spent in the SIZs for each arm to determine if they used social cues (i.e., location in trial 5) or spatial cues (i.e., location in previous trials) to guide investigation behavior. We expected that subjects would spend the majority of their time in the SIZ nearest the novel stimulus animals, since this would presumably be the most salient socio-spatial cue available. Our results showed a main effect for subjects, who spent the majority of their time in the SIZ near the novel stimulus animals ($F_{3, 180} = 8.80$, $P < 0.0001$; Fig 3.2b). Although we found no main effect for time spent in the four SIZs across treatment group ($F_{3, 180} = 0.43$, $P = 0.73$), we did find a significant interaction across treatments and SIZs ($F_{9, 180} = 2.17$, $P = 0.03$; Fig 3.2b). The interaction effect shows that the subjects experiencing the depleted pre-wean and post-wean social environments showed a significantly different pattern of investigation from all other subjects. Specifically, dad absent/single-housed subjects spent the majority of their time in the SIZ where the replaced stimulus animal was

located during trials 1-4, whereas all other subjects spent the majority of their time in the SIZ near the novel stimulus animal (Fig 3.2b).

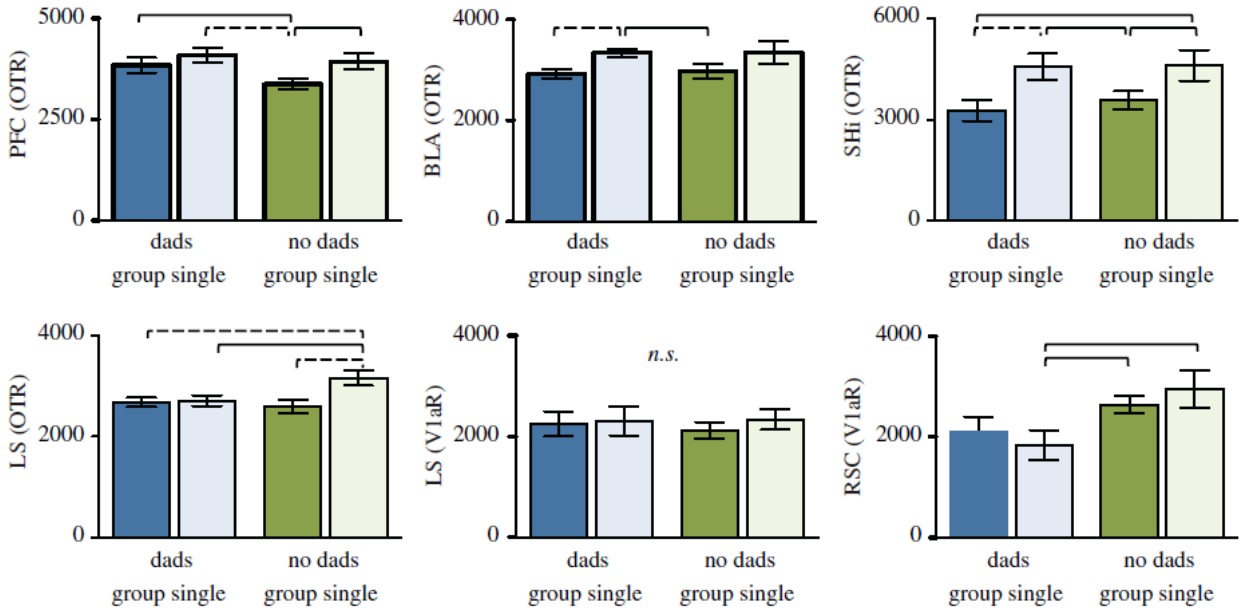
To confirm that this result was not a function of social avoidance, we compared the time dad absent/single-housed subjects spent in the empty arms and the arms containing a conspecific for each presentation trial (trials 1 – 5). In trial 5 (after stimulus animals had been rotated in space) dad absent/single-housed males spent more time in the arms housing no conspecifics ($F_{3, 60}$ = 3.44, P = 0.02; Supplemental Figure S3.1). In contrast, and like all other groups of males, during trials 1 through 4, these males spent more time in arms containing conspecifics (all F's $_{3, 60} \leq$ 1.48, $P's \geq 0.23$; Supplemental Figure S3.1). These data demonstrate that dad absent/singly-housed animals did not avoid social contact in trial 5, but rather that they were using a different search pattern to explore the apparatus after the social and spatial associations had been experimentally disrupted.

Early life social environments and nonapeptide receptor densities

Early life social experience did not affect OTR or V1aR expression in many areas of the pairbonding neural circuit, the social behavior network or the socio-spatial memory structures that we examined (Supplemental Table S3.1). However pre- and post-wean social experience did differentially affect nonapeptide receptor expression in some structures in three interesting ways. First, we found a main effect of pre-wean experience on V1aR density in the retrosplenial cortex (ANOVA: $F_{1, 56}$ = 8.335, P = 0.006; Fig 3.3) and the medial amygdala ($F_{1, 56}$ = 3.936, P = 0.052; Supplemental Table S3.1), with pups without fathers expressing greater V1aR than pups reared with both parents. The effect in the MeA was relatively subtle (Supplemental Figure S3.2).

Secondly, we found that post-wean social experience influenced OTR density in the prefrontal cortex ($F_{1,55} = 5.430$, $P = 0.02$), basolateral amygdala ($F_{1,54} = 6.763$, $P = 0.01$), and septohippocampal nucleus ($F_{1,55} = 10.40$, $P = 0.002$). In all three cases, socially isolated males had higher OTR expression than group-housed males (Fig 3.3). Finally, like the PFC, BLA and SHi, the lateral septum showed a main effect for more OTR among the socially isolated males ($F_{1,56} = 5.575$, $P = 0.02$). However, OTR in the LS also showed an interaction between pre-wean and post-wean social environments ($F_{1,56} = 4.596$, $P = 0.04$; Fig 3.3), in which dad absent/single-housed males expressed greater OTR density than males from all other groups. In other words, the same males that demonstrated deficits in social discrimination also expressed more OTR in the LS. In contrast, we found no main effect for pre-wean experience on LS OTR ($F_{1,56} = 2.177$, $P = 0.15$) and no significant effects of early life social experience on V1aR in the LS (Fig 3.3; Supplemental Table S3.1).

Figure 3.3. Mean (±SE) oxytocin receptor (OTR) and vasopressin 1a receptor (V1aR) density (dpm/mg TE) in the prefrontal cortex (PFC), basolateral amygdala (BLA), septo-hipocampal nucleus (SHi), lateral septum (LS), and retrosplenial cortex (RSC). Colors and shades for treatment groups correspond with Figure 3.2a. Post hoc differences are indicated with solid ($p \leq 0.05$) and dashed ($p \leq 0.01$) lines.



DISCUSSION

Our results show that early- and late-stages of postnatal development in voles interact to impact social discrimination, socio-spatial memory, and the nonapeptide mechanisms that are closely linked with these behaviors. We found that reduced social exposure (i.e., males raised without fathers and later housed alone) interfered with male prairie vole social discrimination and how they approach socio-spatial challenges. We also found that reduced social exposure at different periods of early life can differentially affect nonapeptide expression patterns. While V1aR in the RSC and MeA are altered by the presence or absence of fathers, several oxytocinergic structures (PFC, BLA, LS and SHi) are influenced by post-wean social conditions. Strikingly, OTR, but not V1aR expression in the LS – a structure in which both OT and VP are known to influence social recognition – differed among the same groups of animals that showed deficits in social recognition. Taken together, these results demonstrate that social environments experienced during pre-weaning and post-weaning influence neural development in a region-specific manner, and can interactively shape neural and behavioral phenotype.

Paternal influences on social cognition and development.

Early life social environments can profoundly influence the phenotypic development of an individual. Pioneering work by classic and recent studies has shown that disruption of mother-infant interactions has consequences for the physiology and social behavior of adult offspring (Bale et al., 2010; Champagne & Curley, 2009; Harlow, 1958; Levine, 1957). Despite the significant advances that studies such as these have made, the majority of research performed has focused on maternal care and has overlooked the contribution of fathers on offspring development. In species with bi-parental care, both parents invest heavily in rearing offspring.

Bi-parental care is closely related to adopting a monogamous mating system, something found in only 5% of mammalian species (Kleiman, 1977). Because the occurrence of mammalian paternal care is relatively rare, it is particularly difficult to assess the importance of paternal behavior among mammals, with most work having been conducted in humans. The potential impact the presence of caring fathers has on developing offspring is of significant importance since, among humans for example, it may determine a child's appropriate emotional and cognitive development (Phares & Compas, 1992), health (Flinn & England, 1997), and likelihood to engage in violent activity and crime (Ember & Ember, 1994). On the other hand, a positive paternal influence on developing children increases cognitive competence (e.g., (Forehand & Nousiainen, 1993)) and adult psychological adjustment (e.g., (Amato, 1994)). Non-human work on paternal care is beginning to gain appreciation and it is clear that the role of the father in bi-parental species is significant (Braun & Champagne, 2014). For instance, paternal absence in prairie voles retards development, and can interfere with pairbonding (Ahern & Young, 2009; Z. Wang & Novak, 1994; Z. X. Wang & Novak, 1992). Our results add to this story indicating that the lack of fathers in the postnatal environment also interferes with socio-cognitive behaviors.

Protective influences of post-wean environments on social cognition.

Our results demonstrated that male prairie voles that were raised without fathers showed no evidence for social discrimination, but only if they later lived in social isolation. These results are unique in that they highlight a particularly compelling role for social housing in later life, which may act as a social buffer to protect animals from demonstrating socio-cognitive deficits that might result from reduced paternal care. Although the combination of environmental and social enrichment is known to reverse the influence of impoverished mother-pup interactions

(Bredy et al., 2003; Bredy et al., 2004; Champagne & Meaney, 2007), these studies were unable to directly attribute their effects to either social or physical enrichment. To our knowledge our study is the first demonstration of a cognitive deficit that is potentially both caused and rescued by social context alone.

Furthermore, the roles of OT and VP acting in the LS on social recognition are well established (Bielsky & Young, 2004; Bohus et al., 1978; Ferguson et al., 2002; Wacker & Ludwig, 2012). Septal VP facilitates social recognition, while endogenous levels of OT inhibit social recognition. Relatively elevated levels of OTR in the LS should therefore increase animals' sensitivity to the inhibitory effects of OT on social recognition. The fact that males raised without fathers and later housed alone demonstrated both deficits in social discrimination and greater OTR density suggests that continued social deprivation throughout early life affects septal OTR phenotype, which in turn affects social recognition. A similar pattern was not evident in septal V1aR, which was surprising.

Did males with the greatest social experience also show recognition deficits?

Based on our data, it was unclear if males raised with fathers and then group housed (arguably males with the most social exposure) showed evidence for social discrimination or not. Although this is a peculiar result, we note that this group did show a non-significant trend toward showing social discrimination, and group performance was more similar to the groups that clearly showed recognition than the one group that clearly did not (Fig 3.2a). This result might better represent an underpowered sample rather than a lack of evidence for social recognition. We have repeatedly demonstrated that animals raised with fathers and then group housed show social

recognition (Zheng, Foley, Rehman, & Ophir, 2013). Moreover, we showed testing animals in contexts that lack preexisting social cues (i.e., clean testing apparatus) produces high variance in social recognition. Similarly, dad present/group-housed males showed the greatest variation of all four treatment groups in the current study, a result primarily driven by three animals. It is possible that these animals were particularly affected by the lack of social cues in the testing apparatus. Removal of any one of these three animals from the analyses would have produced a significant result. Taken together, we feel that this result is most likely a sampling error and not evidence for lack of recognition.

Early life social experience influences how environmental cues are used.

Differences resulting from social deprivation spanning postnatal and sub-adult periods of development extended beyond social discrimination. Indeed these animals also demonstrated distinct ways of using environmental information. Whereas most animals appeared to focus on social cues to guide behavior in the socio-spatial memory test, animals experiencing reduced social exposure appeared to rely more heavily on spatial cues. It is hard to interpret why these animals appeared to preferentially visit the arm where a familiar animal that can no longer be located used to be, however it is clear it was not a social aversion (Supplemental Figure S3.1). Furthermore, all animals were able to locate novel and familiar conspecifics in the experiment during the test period; animals in all groups spent some proportion of time in every arm (SIZ) of the maze. One interpretation of these results is that animals experiencing reduced social exposure are less able to resolve broken associations between spatial location and social identity. This notion is consistent with the performance of these animals, in which they spent similar, relatively low, amounts of time investigating arms containing stimulus animals (either familiar or

unfamiliar) in the test trial, but returned throughout this period to the arm that formerly contained an animal that was nowhere to be found. Another possibility is that the social search strategies of male prairie voles are influenced by postnatal social experiences, producing differential motivation to either engage with novel animals or account for all conspecifics (particularly ones that have gone missing). If this is true, then the relatively poor performance in social discrimination that these animals showed might be explained by an altered social search strategy rather than actual deficiency in social discrimination. However, we think this is unlikely considering that the deficits in social recognition could be accounted for by a well supported proximate explanation: the tight link between septal OT/OTR action and its role in disrupting social discrimination combined with the powerful observation that the same group of animals that showed recognition deficits also showed elevated OTR expression (see section above). Nevertheless, deficient social recognition could very well contribute to an altered search strategy. A third possibility is that postnatal social experiences alter the weighting that male prairie voles give to spatial or social cues. In other words, perhaps animals experiencing reduced social exposure are more attentive to spatial cues over social cues, thereby demonstrating an over-reliance on spatial cues. Further studies are clearly necessary to explain the nature of this difference in cognitive behavior.

Nonapeptide receptor sensitivity to developmental social environments.

Results from our autoradiographic analyses showed several intriguing outcomes. First we found that nonapeptide receptor expression is relatively robust throughout postnatal development. The presence or absence of fathers only affected receptor expression in a handful of structures. The expression patterns of V1aR in the RSC and MeA were higher in males raised without fathers.

On the other hand, social isolation after weaning was associated with greater expression of OTR in the PFC (an area of particular importance for pairbonding), the BLA (a central part of the social behavior network involved in emotional processing and valuation), the LS (an area already noted for its importance in pairbonding and social recognition, and that is part of the social behavior network), and the SHi (an area that relays between the hippocampus and lateral septum and is known to be important in socio-spatial memory). This general influence of neonatal and juvenile social experience in V1aR and OTR (respectively) suggests that nonapeptide receptor phenotype is sensitive to the social environment across several inter-related neural circuits and systems.

Can early life social experience shape reproductive success among mating tactics?

Prairie voles are socially monogamous, and have received much notoriety for their usefulness in understanding the mammalian neurobiology that underlies social attachment. An occasionally overlooked aspect of their natural history is that while a majority of males and females form bonds and establish socially monogamous breeding units ('residents'), an important minority of prairie voles remains single and traverses large undefended home ranges ('wanderers') (Getz et al., 1993; McGuire & Getz, 2010; McGuire et al., 2013; Ophir, Phelps, et al., 2008). Although the V1aR and OTR profiles of residents and wanderers do not differ among structures involved in the 'pairbonding neural circuit', structures important for socio-spatial memory robustly predict whether residents or wanderers sire offspring (Ophir et al., 2012; Ophir, Wolff, et al., 2008). Specifically, these studies revealed an interaction between mating tactic (resident / wanderer) and reproductive success (sired offspring or did not), with reproductively unsuccessful wanderers having significantly more nonapeptide receptors than successful wanderers in key socio-spatial

memory neural structures, in particular RSC and SHi (Ophir et al., 2012; Ophir, Wolff, et al., 2008). These and other results suggest socio-spatial memory is important in shaping mating tactics (Ophir et al., 2012; Ophir, Wolff, et al., 2008; Ophir et al., 2009; Zheng, Foley, et al., 2013; Zheng, Larsson, et al., 2013).

In the current study, differences in RSC V1aR were attributed to the presence or absence of fathers and differences in SHi OTR were shaped by the post-wean social environment. Interestingly, the combination of these two effects produced a particularly striking outcome for the individuals experiencing reduced social exposure throughout development. These males had both high V1aR in the RSC and high OTR in the SHi, which recapitulates a major component of the unique phenotype exhibited by the wanderers that did not sire offspring in the field (Ophir et al., 2012; Ophir, Wolff, et al., 2008). Although many other mechanisms are sure to be involved, this result raises the provocative possibility that reduced social interaction during both pre- and post-wean development might contribute to a neural phenotype that disadvantages wanderers under natural conditions. This idea is clearly speculative and merits further testing.

Conclusion.

We have shown that socio-cognitive development and neural phenotype are susceptible to the influences of early life social environments. Some studies have demonstrated that pre-weaning environments have the potential to shape adult pro-social behavior, thereby facilitating the establishment of relationships (e.g., attachment, and affiliation; (Ahern & Young, 2009)). Meanwhile, the post-weaning environments may influence stress reactivity and other aspects of behavior that may modulate anti-social behaviors (Grippo et al., 2007; Lukkes et al., 2009). Such

experiences have been suggested as means for informing and preparing individuals to survive when environments that they are likely to experience as adults are dynamic, variable, or relatively unpredictable (Champagne & Meaney, 2007). The relative survival value that socio-spatial cognition may have extends the potential importance of early life social experiences on relevant neural and behavioral mechanisms. We have long appreciated that quality and quantity of parental care has long-term implications for adult behavior (e.g., (Harlow, 1958; Levine, 1957)). Our results indicate that subsequent social experiences can serve to protect and even potentially rescue aspects of social cognition in individuals that experience social adversity early in their lives. In addition to promoting survival and social relationships, the combination of childhood and adolescent social experiences may predispose individuals to successfully navigate the reproductive challenges that await them in adulthood.

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SUPPLEMENTAL MATERIAL

Supplemental Table S3.1. Differences in V1aR and OTR density across neural areas associated with pairbonding, the social behavior network, and socio-spatial memory.

	Pre-wean		Post-wean		Interaction	
V1aR *(df)*	F	*P*	F	*P*	F	*P*
VPall *(1, 56)*	0.04	0.83	0.63	0.43	0.82	0.37
LS *(1, 56)*	0.08	0.78	0.40	0.53	0.15	0.70
BNST *(1, 56)*	0.03	0.87	1.02	0.32	1.70	0.20
AH *(1, 56)*	0.05	0.82	0.15	0.70	0.47	0.50
RSC *(1, 56)*	***8.13***	***0.006***	0.007	0.93	1.00	0.32
CeA *(1, 56)*	0.78	0.38	0.001	0.98	0.54	0.47
MeA *(1, 56)*	***3.94***	***0.05***	0.03	0.86	0.36	0.55
VMH *(1, 56)*	0.67	0.41	0.63	0.43	0.12	0.73
OTR *(df)*	F	*P*	F	*P*	F	*P*
PFC *(1, 55)*	3.25	0.08	***5.43***	***0.02***	0.76	0.39
NAcc *(1, 55)*	0.007	0.93	0.60	0.44	2.25	0.14
SHi *(1, 55)*	0.23	0.63	***10.40***	***0.002***	0.13	0.72
LS *(1, 56)*	2.18	0.15	***5.58***	***0.02***	***4.57***	***0.04***
Hi *(1, 54)*	0.50	0.48	1.40	0.24	0.12	0.74
CeA *(1, 54)*	1.59	0.21	0.71	0.40	1.59	0.21
BLA *(1, 54)*	0.05	0.83	***6.76***	***0.01***	0.02	0.90

Two-factor ANOVA results for V1aR (^{125}I-Vasopressin) and OTR (^{125}I-Ornathine Vasotocin) binding in disintegrations per minute per mg in rat brain tissue equivalence (dpm/mg TE). Factors were based on early life social experience: Pre-wean (dads present or absent) and post-wean (group or single housed). Sample sizes for all V1aR expressing structures were N = 15 for dad present/group-housed (P-G), N = 14 for dad present/ single-housed (P-S), N = 16 for dad absent/group-housed (A-G), and N = 15 for dad absent/ single-housed (A-S). Sample sizes for OTR varied by structure: *PFC*: P-G [N = 13], P-S [N = 14], A-G [N = 14], A-S [N = 14]; *NAcc*: P-G [N = 13], P-S [N = 14], A-G [N = 14], A-S [N = 14]; *SHi*: P-G [N = 14], P-S [N = 13], A-G [N = 14], A-S [N = 14]; *LS*: P-G [N = 14], P-S [N = 14], A-G [N = 14], A-S [N = 14]; *Hi*: P-G [N = 13], P-S [N = 14], A-G [N = 13], A-S [N = 14]; *CeA*: P-G [N = 13], P-S [N = 14], A-G [N = 13], A-S [N = 14]; *BLA*: P-G [N = 13], P-S [N = 14], A-G [N = 13], A-S [N = 14]. Abbreviations are defined in text. Significant *P*-values are in bold.

Supplemental Figure S3.1. Effects of rearing condition on time spent in social inspection zones across five trials of the socio-spatial memory test.

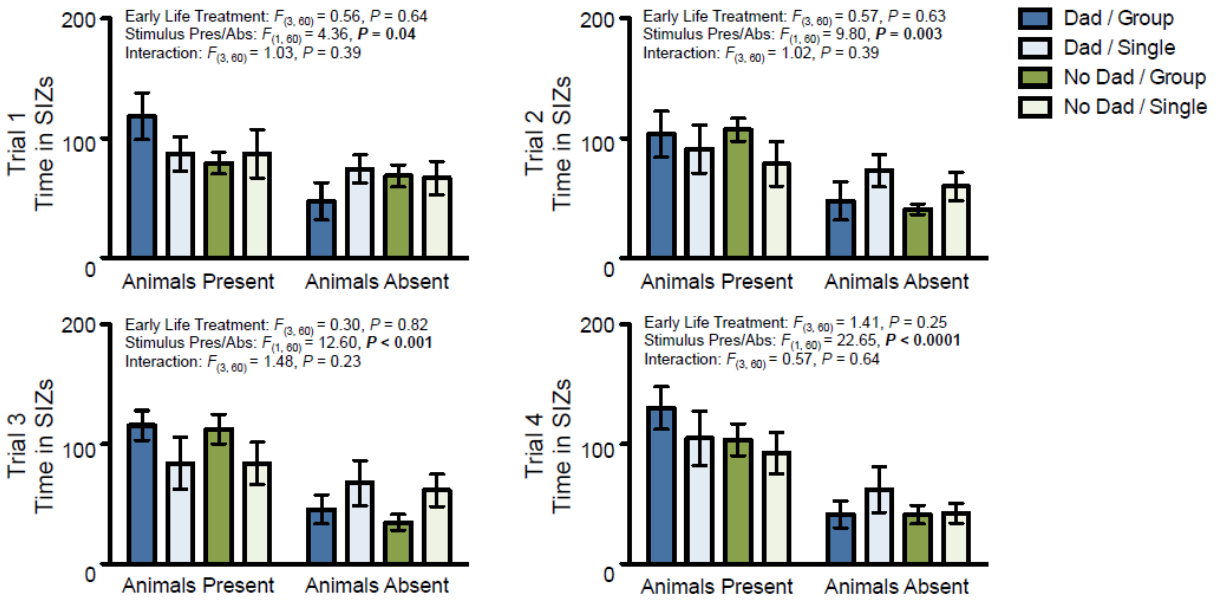
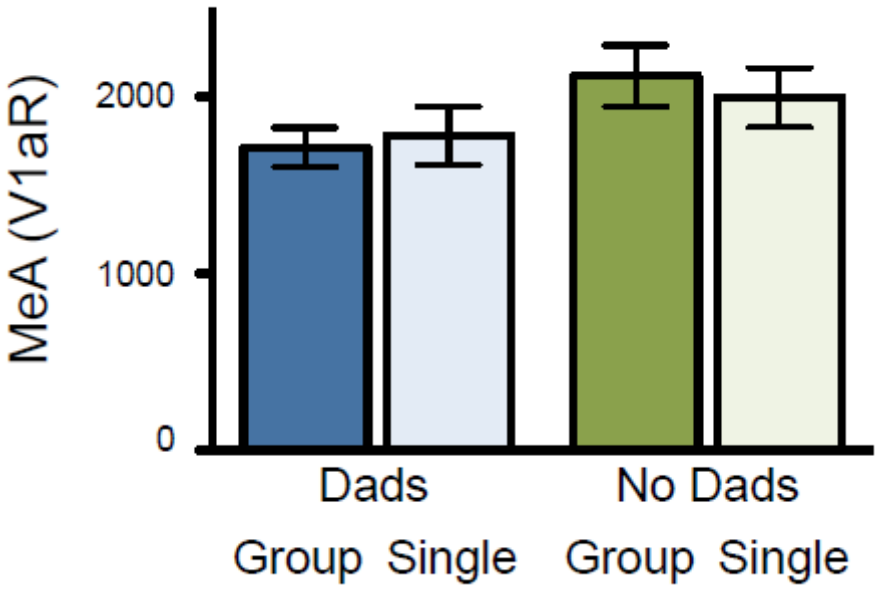


Figure S1. Mean (±SE) time (in seconds) animals from each rearing condition spent in social inspection zones (SIZ) containing stimulus animals (animals present) or that were empty (animals absent) for each of the five trials during the socio-spatial memory test. Times from the two arms containing or not containing an animal were collapsed. Blue bars indicate that males were reared with fathers postnatally; green bars indicate that males were reared without fathers. Dark shading indicates subjects were raised in groups after they were weaned; light shading indicates subjects were housed alone after weaning. Results from two factor ANOVA are presented for each panel, and demonstrate that across treatment groups, subjects spent more time in arms containing stimulus animals. More importantly, the data show that the socially deprived subjects (raised without fathers and housed alone; light green) did not differ in the time they spent in the social inspection zones containing stimulus animals in trials 1 through 4. This indicates that this group of animals was not avoiding social contact in trials 1 – 4 any more than subjects from the other rearing conditions, supporting the hypothesis that these animals demonstrate a different pattern of reliance on socio-spatial cues after the location of stimulus animals has been moved (trial 5).

Supplemental Figure S3.2. Mean (±SE) vasopressin 1a receptor (V1aR) density in the medial amygdala (MeA).



CHAPTER 4 – The impact of perinatal and juvenile social environments on the effects of chronic intranasal oxytocin in the prairie vole

George S. Prounis and Alexander G. Ophir

ABSTRACT

Over the course of development social environments can interact with neuroendocrine mechanisms to dictate changes in behavior. Experiences with parents and peers during perinatal and pre-adult stages of development can shape the expression of oxytocin in the brain, and thereby affect social behavior. We investigated whether the effect of social environments on the neural oxytocin system would impact the influence of chronic intranasal oxytocin on social behavior at different stages of development. We raised male prairie voles under two conditions: either they were bi-parentally reared and then weaned into group housing (BP+GRP), or reared by a single-mother, and that then weaned into social isolation (SM+ISO). Males raised under these conditions were either exposed to repeated (daily) doses of intra-nasal oxytocin (IN-OT) or a saline control for twenty-one days from postnatal day 21 to 42. We found that SM-ISO males, exhibited increased social contact in Juvenile Affiliation tests at postnatal day 35 and 42. These SM+ISO males were also more likely to form a partner preference than BP+GRP males, and formed stronger partner preferences overall. IN-OT did not alter these behavioral effects. Additionally, we demonstrated that SM+ISO males exhibited a distinct response to non-invasive chronic treatment with exogenous oxytocin. When compared to all other treatment groups, SM+ISO males that received intranasal oxytocin exhibited a greater amount of huddling behavior in an alloparental care test. This effect was, in part, explained by an absence of attack behavior, found only in these males. This study contributes to understanding the complex interactions between social environment, nonapeptide systems, and social behavior. In addition, our results hold translational merit, highlighting a mechanism of socioenvironmental influence over behavioral response to intranasal oxytocin.

INTRODUCTION

Social environments can vary across stages of development and shape the social behavior of an individual. Plasticity of nonapeptide systems, such as oxytocin (OT) and its receptor (OTR), offer plausible mechanisms for translating shifts in social environment to changes in social behavior (Bales & Perkeybile, 2012). During defined windows of development, social environments may mediate changes to OT synthesis and/or OTR expression. These windows of development are characterized by specific features of the social environment that can impact these nonapeptide systems and alter social behavior.

Interactions with parents during perinatal stages of life can shape the development of social behavior and OT systems. Rats that receive higher amounts of maternal care develop increased densities of OTR in specific brain regions and exhibit higher maternal care behavior as adults (F. Champagne, Diorio, Sharma, & Meaney, 2001; Francis, Champagne, & Meaney, 2000; Francis, Young, Meaney, & Insel, 2002). On the other hand, rats experiencing maternal separation express region-specific increases or decreases of OTR and exhibit increases in anxiety and aggressive behavior (Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Lukas, Bredewold, Neumann, & Veenema, 2010; Veenema, 2009). In bi-parental species, such as the mandarin vole and prairie vole, removal of fathers from the family unit can alter OTR development and induce impairments to social cognition (Cao et al., 2014; Prounis, Foley, Rehman, & Ophir, 2015), and this can also reduce alloparental care behavior and partner preference behavior (Ahern & Young, 2009).

Social factors beyond the natal environment can also shape nonapeptide systems and behavior

during juvenile and adolescent development. Male prairie voles that live in a socially and spatially enriched environment after weaning develop higher densities of OTR in many regions of the forebrain (Prounis, Thomas, & Ophir, 2018). On the other hand, social isolation during pre-adult stages can increase aggressive behavior in rats (Wongwitdecha & Marsden, 1996), and affect OTR receptor density (Prounis et al., 2015) and promote anxiety (Pan, Liu, Young, Zhang, & Wang, 2009) and depressive-like behaviors (Grippo, Gerena, et al., 2007) in prairie voles. Social environments during perinatal and pre-adult stages of development have even been shown to interact. The quality of maternal care behavior received during perinatal development and environmental enrichment during pre-adult development interactively shape OTR expression and maternal behavior in rats (F. A. Champagne & Meaney, 2007). We have shown that male prairie voles that are reared by a single mother and then later experience isolated housing, demonstrate region-specific increases in OTR and an impairment to social recognition (Prounis et al., 2015).

Manipulations using exogenous OT in early development affect social behavior later in life. Intraperitoneal (i.p.) injections of OT in neonatal prairie voles lead to dose-specific changes to care behavior and attachment behavior (Bales et al., 2007). Oxytocin i.p. injections reverse the effect of social isolation on depressive-like behaviors (e.g., helplessness and anhedonia; (Grippo, Trahanas, Zimmerman, Porges, & Carter, 2009)) but not anxiety (Grippo et al., 2012). A recent body of research suggests that intranasal OT (IN-OT) provides a non-invasive means to alter central levels of extracellular oxytocin in rodents (Neumann, Maloumby, Beiderbeck, Lukas, & Landgraf, 2013). Behavioral studies in rodents suggest that IN-OT treatment can lead to changes in social behavior. In prairie voles, chronic IN-OT treatment increases social contact with sibling cagemates (Bales et al., 2013). Medium and low doses of IN-OT, but not a large dose, impair

partner preference behavior in male prairie voles (Bales et al., 2013). Changes in behavior may be mediated by the effects of IN-OT on OTR expression (Guoynes et al., 2018).

We explored the dynamic interaction between perinatal social environment, pre-adult social environment, and chronic dosing of extracellular OT via intranasal application on social behavior of the prairie vole at various stages of development. A few characteristics of prairie voles make the species particularly useful for the study of interactions between social context, social behavior, and OT. First, prairie voles experience a wide variety of social experiences early in life ranging from single-mother rearing, bi-parental rearing, and communal rearing in nature (Getz, Carter, & Gavish, 1981). Second, prairie voles develop social behaviors across life history that are distinct when compared to other rodent species: juvenile prairie voles readily engage in care behavior towards novel unrelated pups ('spontaneous alloparental care';(Roberts, Miller, Taymans, & Carter, 1998)), and adult prairie voles can form social attachments with opposite sex conspecifics after cohabitation or mating ('partner preference behavior';(Getz et al., 1981; Williams, Catania, & Carter, 1992)). Lastly, these social behaviors have been shown to be causally linked to OT function in the brain (Liu & Wang, 2003; Olazabal & Young, 2006). In the current study, we hypothesized that the interaction between perinatal social environments (single-mother reared versus bi-parentally reared) and pre-adult social environments (isolated housing versus group housing) would result in distinct expressions of prosocial behavior during adolescence and adulthood. We predicted that intranasal oxytocin treatment will increase the prosocial behavior of socially deprived prairie voles to a greater extent than voles experiencing standard rearing. This prediction is based on previous findings from our lab that showed socially deprived voles exhibit higher densities of OTR in regions of the brain implicated in prosocial

behavior (i.e., the lateral septum, prefrontal cortex, and basolateral amygdala) (Prounis et al., 2015). Our reported findings have translational value, providing evidence that social environments can influence prosocial responses to intranasal oxytocin treatment.

METHODS

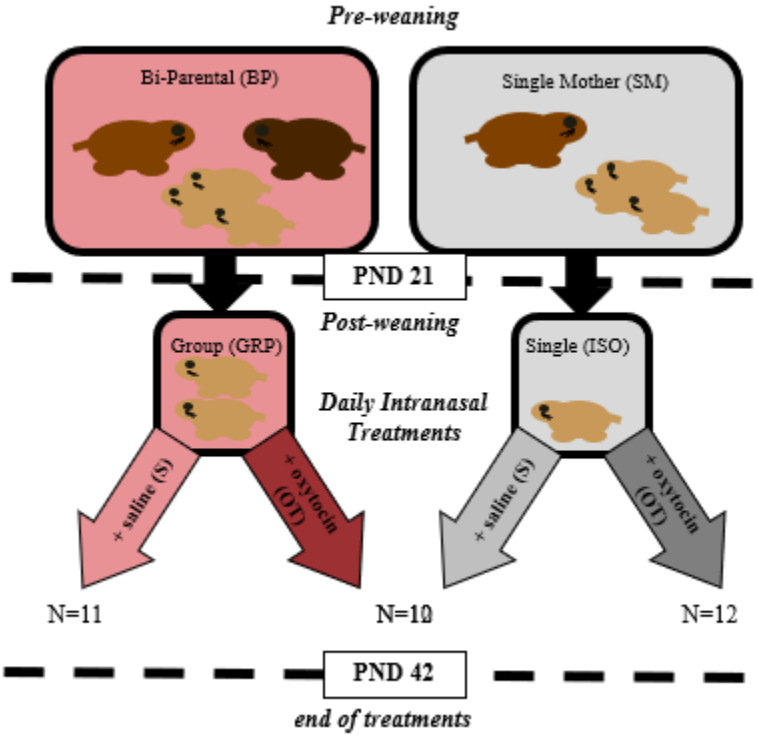
Animals and early life manipulations

All subjects came from the first litter of breeding pairs created specifically for this experiment. The breeders originated from our colony of prairie voles, which were originally trapped in Champaign County, IL, USA. Animals were housed in standard polycarbonate rodent cages (29 x 18 x 13 cm) lined with Sani-chip bedding and provided nesting material and kept on a 14 L: 10 D cycle. Animals were provided with rodent chow (Laboratory Rodent Diet 5001, LabDiet, St. Louis MO, USA) and water *ad libitum*. Ambient temperature was maintained at 20 +/- 2 C°. All procedures were approved by the Institutional Care and Use Committee of Cornell University (protocol number 2013-0102).

All breeding units created for this experiment had litters culled to 3-5 pups. Male subjects were assigned to groups at birth that exposed them to two different social experiences across pre-weaning and post-weaning development. One group of males was reared by both a mother and father (i.e., a biparental family unit). At weaning (PND 21) these same males were placed in housing with a male sibling (Fig 4.1, BP→GRP). The second group of males was reared by a single mother after the father was removed on postnatal day (PND) 0. At weaning (PND 21) these same males were housed in isolation (Fig 4.1, SM→ISO). We have previously shown that these manipulations during both pre-weaning and post-weaning produce group differences in

OTR expression and social cognition in male prairie voles (Prounis et al., 2015).

Figure 4.1 Timeline of environmental manipulations, intranasal treatment, and group assignments.



Intranasal oxytocin treatments

Between PND 21-45 subjects received daily intranasal treatments of either saline (S) or oxytocin (OT; 0.8 IU/kg) between 0800h-1200h (Fig 4.1). This dose of chronic intranasal oxytocin treatment impacts social behavior of male prairie voles and is closely equivalent to a weight-adjusted dose commonly used in human studies (Bales et al., 2013). We applied a total of 25ul of saline or OT from a pipette tip around the nasal cavity while the subject was scruffed and held upside down, alternating sides so that 12.5ul was applied to each nostril and resulting in

inhalation of the solution. In total, four groups were created corresponding with both early life manipulation and intranasal treatment: BP→GRP+S, BP→GRP+OT, SM→ISO+S, SM→ISO+OT (Fig 4.1). Subjects were weighed every week to determine the effects of the different conditions on body size and growth, and to factor any mass differences into performance on behavioral tests.

Behavioral Testing

Subjects performed a battery of behavioral tests during and after the period of intranasal treatment. These included a series of four Juvenile Affiliation tests, two Alloparental Care test, and a Partner Preference test (see below).

Juvenile Affiliation test – Immediately after intranasal treatment on PND 22, 28, 35, and 42, subjects were placed in a standard sized cage (29 x 18 x 13 cm) for thirty minutes of acclimation prior to testing. After acclimation, unrelated and novel juvenile voles (between PND 15-21 of age) were placed on the opposite end of the cage from the subject. We assessed the social contact time (defined as all non-agonistic physical contact) between subjects and the juvenile voles during a 10-minute 'Juvenile Affiliation test'. The timing of these tests corresponded with expected release of oxytocin in the brain after intranasal treatment (Neumann et al., 2013). Thus, the weekly Juvenile Affiliation tests were intended to examine the immediate effects of intranasal oxytocin treatment on prosocial behavior with a nonthreatening stimulus animal, while also testing for changes in social responses over the course of chronic administration. The final sample size analyzed for all four Juvenile Affiliation tests was: BP→GRP+S = 11, BP→GRP+OT = 10, SM→ISO+S = 12, SM→ISO+OT = 12.

Alloparental Care tests - On the day after the last intranasal treatment (PND 43), subjects performed the first of two Alloparental Care tests. Subjects were placed in a novel standard sized cage (29 x 18 x 13 cm) to acclimate for thirty minutes. After acclimation, two unrelated neonates (between PND 2-5 of age) were placed at the opposite end of the cage from the subject. The amount of huddling behavior and aggression was quantified during the 10-minute test. Huddling behavior was scored as the duration of time the subject was stationary and completely covering at least one of the stimulus pups. Aggression was scored as any lunges and biting behavior. An experimenter watched the social interaction from a close distance to ensure that if the subject behaved aggressively to the stimulus pup, the trial could be terminated before the stimulus animal was harmed. If the stimulus animal showed bite marks on their skin after an attack, they were immediately euthanized humanely. A second Alloparental Care test was implemented on PND 58, as just described. This allowed us to determine behavioral effects both immediately and weeks after the period of chronic intranasal treatment. This schedule also allowed us to examine the effects of treatment on behavior at an age associated with sub-adult peripubertal animals (~ PND 45) and adulthood (~PND 60). There were two instances where the subject did not move from the corner of the cage at the start of the video (one during the PND 43 test, and one during the PND 58 test); these recordings were excluded from analysis. The final sample size analyzed for the PND 43 Alloparental Care test was: BP→GRP+S = 11, BP→GRP+OT = 10, SM→ISO+S = 12, SM→ISO+OT = 11. The final sample size analyzed for the PND 58 Alloparental Care test was: BP→GRP+S = 11, BP→GRP+OT = 9, SM→ISO+S = 12, SM→ISO+OT = 12.

Partner Preference test - Lastly, on PND 60 subjects performed a Partner Preference test after 24 hours of cohabitation with a sexually receptive female primed with dirty male bedding (Carter, Getz, Gavish, McDermott, & Arnold, 1980; Dluzen, Ramirez, Carter, & Getz, 1981; Richmond & Stehn, 1976). To prime the females, urine-soaked bedding from the cage of males that were unrelated to the male subject and the female was placed in the female's home cage a day before animal pairing. The 24 hour cohabitation time has been shown to be sufficient to form partner preference in male prairie voles. On the day of testing, the female partner and the unfamiliar female were tethered to opposite compartments of a three-compartment apparatus. After 30 minutes of acclimation, the subject male was placed in the unoccupied middle compartment from which it could freely move between all three compartments of the apparatus. An observer blind to treatment scored the amount of time the subject spent in side-by-side contact with both the female partner and with an unfamiliar sexually primed adult female over a 180-minute test period. These times were compared to determine the degree of preference to cohabitate with the partner. Two recordings were unable to be analyzed due to technical issues involving trimming of the original camera files. The final sample size analyzed for the Partner Preference test was: BP→GRP+S = 11, BP→GRP+OT = 9, SM→ISO+S = 11, SM→ISO+OT = 12.

Data analysis

All behavioral data were collected using Noldus Observer. For the Juvenile Affiliation tests, we performed a two-factor ANOVA to compare social contact time between groups according to social manipulation and intranasal treatment, and we used Tukey HSD tests to determine significant post-hoc comparisons between groups. Due to non-normal distribution of data for proportion of huddling time in the Alloparental Care tests, we performed a Kruskal-Wallis test to

detect significant group differences, and we used Dunn's test for post-hoc comparisons between groups. We performed a Pearson's chi-square test for independence to compare the incidence of attack behavior in the Alloparental Care tests. We believe the attack behavior, in representing an absolute absence of care behavior, is a critical component in understanding the group differences in alloparental care behavior. Excluding subjects that attacked the pups from analysis of the Alloparental Care test would also severely reduce our sample size and result in a drastic loss of statistical power. For these reasons, we included subjects that attacked pups in the analysis of huddling time. Paired t-tests compared the side-by-side contact time with a partner female versus an unfamiliar female for every group in the Partner Preference test. A two-factor ANOVA compared the preference score (contact with partner – contact with unfamiliar female) according to social manipulation and intranasal treatment, and Tukey HSD determined significant post-hoc comparisons between groups. We performed a Pearson's chi-square test for independence to compare the proportion of subjects displaying a partner preference in the Partner Preference test. We used Pearson correlations to determine the relationship between body mass and test performance at different stages of development. We considered an alpha ≤ 0.05 to be statistically significant for all tests, rounding all *p*-values to the nearest one-hundredth decimal place.

RESULTS

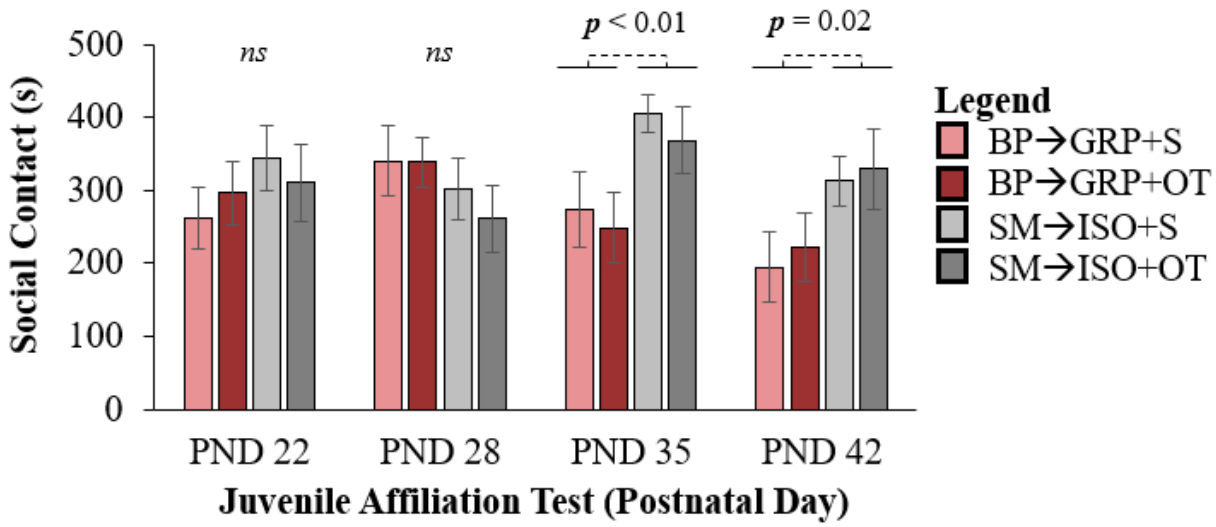
Body mass

SM→ISO males weighed less than BP→GRP males at PND 28 (ANOVA: $F_{1,41} = 4.21$, $p < 0.05$), PND 35 ($F_{1,41} = 4.82$, $p = 0.03$), and PND 42 ($F_{1,41} = 4.73$, $p = 0.04$) (Supplemental Table S4.1). Intranasal OT treatment did not affect body mass at any stage of development. Post-hoc comparisons showed no significant difference in body mass between any combination of the four groups (Tukey HSD: all $p > 0.18$). Body mass did not correlate with any behavioral test (Supplemental Figure S4.1a-g).

Juvenile Affiliation Tests

Social rearing environment did not impact the amount of social contact time with juveniles at PND 22 (ANOVA: $F_{1,41} = 1.10$, $p = 0.30$) and PND 28 ($F_{1,41} = 1.77$, $p = 0.19$) (Fig 4.2). However, we found a main effect of social environment for tests on PND 35 ($F_{1,41} = 8.12$, $p < 0.01$) and PND 42 ($F_{1,41} = 5.86$, $p = 0.02$), with SM→ISO males having more social contact time than BP→GRP males (Fig 4.2). There was no effect of intranasal treatment on social contact with juveniles at any age (PND 22: $F_{1,41} = 0.001$, $p = 0.98$; PND 28: $F_{1,41} = 0.27$, $p = 0.60$; PND 35: $F_{1,41} = 51$, $p = 0.48$; PND 42: $F_{1,41} = 0.22$, $p = 0.64$). Likewise, there were no significant interactions between social environment and intranasal treatment at any age (PND 22: $F_{1,41} = 0.53$, $p = 0.47$; PND 28: $F_{1,41} = 0.20$, $p = 0.66$; PND 35: $F_{1,41} = 0.02$, $p = 0.90$; PND 42: $F_{1,41} = 0.01$, $p = 0.92$). Post-hoc comparisons showed no differences between any of the four groups (Tukey HSD: all comparisons $p>0.08$).

Figure 4.2. The effects of early life social environment and intranasal treatment on juvenile affiliation behavior between weaning and puberty.



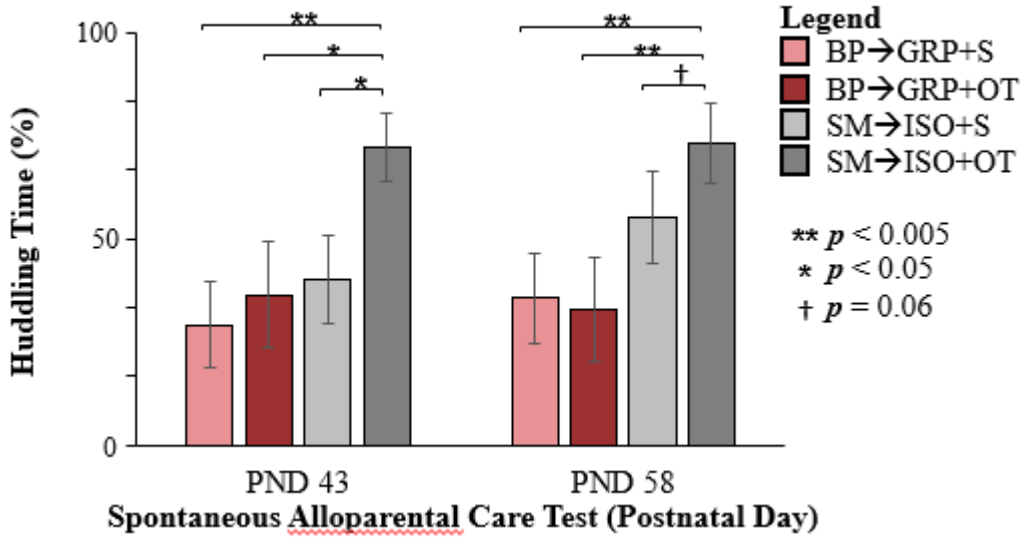
Alloparental Care Tests

We found group differences in the proportion of time subjects huddled with pups at PND 43 in the first Alloparental Care test (Kruskal-Wallis: $x^2(3) = 8.91$, $p = 0.03$). Post-hoc comparisons showed that SM→ISO+OT males spent more time huddling than SM→ISO+S (Dunn's test: $p = 0.01$), BP→GRP+S ($p < 0.005$), and BP→GRP+OT males ($p < 0.02$) (Fig 4.3). We found nearly the same group difference in the PND 58 Alloparental Care test ($x^2(3) = 10.60$, $p = 0.01$), with post-hoc comparisons showing that SM→ISO+OT males spent more time huddling with pups than BP→GRP+S ($p < 0.005$), and BP→GRP+OT males ($p < 0.005$) (Fig 4.3). A non-significant trend suggested SM→ISO+OT males might also spend more time huddling pups than SM→ISO+S males at PND 58 ($p = 0.06$) (Fig 4.3).

At PND 43, immediate attack of pups during the test resulted in early termination of the test with no proportion of time spent huddling scored for a subset of BP→GRP+S males (N=5, 45.5%),

BP→GRP+OT males (N=2, 20%) and SM→ISO+S males (N=3, 25%). Similar incidents of attack behavior were found at PND 58 (BP→GRP+S: N=4, 36.4%; BP→GRP+OT: N=1, 11.1%; SM→ISO+S: N=2, 16.7%). At both PND 43 and PND 58, no SM→ISO+OT males attacked the pups. When we compared all four groups in a chi-square analysis, we found a non-significant trend that hinted toward differences in attack behavior at PND 43 ($x^2(1) = 6.55$, $p = 0.09$; Supplementary Table S4.2a) but not at PND 58 ($x^2(3) = 5.87$, $p = 0.12$; Supplementary Table S4.2b). When comparing groups according to intranasal treatment alone, however, subjects receiving IN-OT were less likely to attack than subjects receiving saline at PND 43 ($x^2(1) = 3.99$, $p = 0.05$; Supplementary Table S4.2c) and PND 58 ($x^2(1) = 3.73$, $p = 0.05$; Supplementary Table S4.2d).

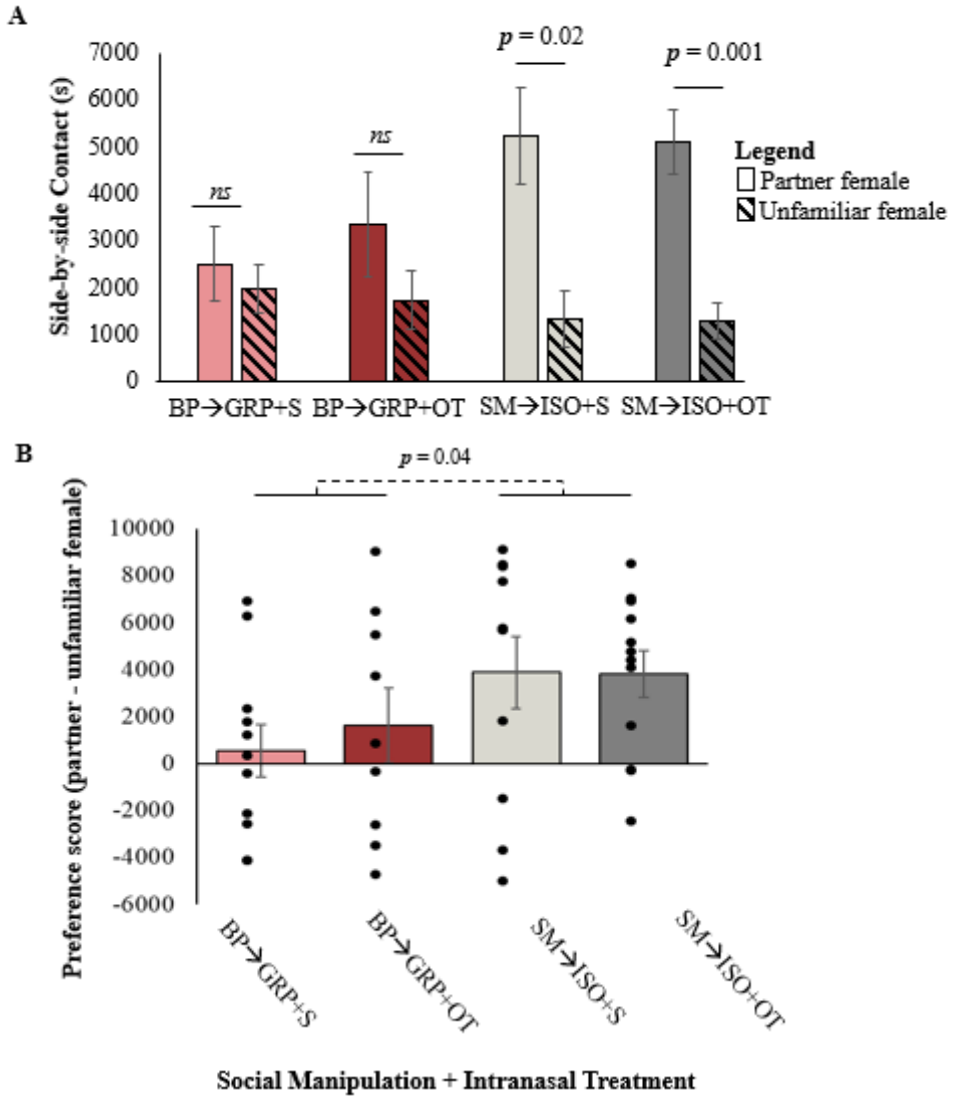
Figure 4.3. The effects of social environment and intranasal treatment on social contact with a novel juvenile across pre-adult stages of development.



Partner Preference Test

We found a significant preference for the female partner only in SM→ISO+OT based on side-by-side contact time with each female in the Partner Preference test ($t(11) = 3.89$, $p = 0.001$) and SM→ISO+OT males ($t(10) = 2.53$, $p = 0.02$) (Fig 4.4a). A preference for side-by-side contact with the partner was not found for BP→GRP+S ($t(10) = 0.48$, $p = 0.32$) and BP→GRP+OT males ($t(8) = 1.02$, $p = 0.17$). Comparison of difference scores of side-by-side contact (time with partner – time with unfamiliar female, a proxy for partner preference) revealed a main effect of social environment, with SM→ISO males having larger difference scores than BP→GRP males (ANOVA: $F_{1,39} = 4.68$, $p = 0.04$; Fig 4.4b). An alternative way to compare the group differences in partner preference behavior accounted for the proportion of individuals demonstrating a preference for the partner, defined as the subject spending over 60% of total side-by-side contact with the partner. According to this approach SM→ISO males (both "+OT" and "+S") were more likely to form a preference than BP→GRP males (both "+OT" and "+S") (chi-square test of independence: $x^2(1) = 3.74$, $p = 0.05$; Supplementary Table S4.3a). However, we found no significant difference in the likelihood of forming a partner preference when considering all four groups independently in a chi-square test ($x^2(3) = 3.76$, $p = 0.29$; Supplementary Table S4.3b).

Figure 4.4. The effects of social environment and intranasal treatment on partner preference behavior. A: Mean side-by-side contact with the female partner and unfamiliar female in the Partner Preference test. B: Preference score (contact with partner – contact with unfamiliar female), with a histogram of group means overlaid with individual scores (filled circles). Error bars = ±SE.



DISCUSSION

The combination of social context and IN-OT had variable effects depending on the developmental stage and the social behavior being tested. The rearing treatment groups contrasted a relatively high socially enriched environment with a relatively low socially depleted environment. We found that socially depleted environments (SM→ISO) produced males with lower body mass, longer durations of social contact time in Juvenile Affiliation tests, and that were more likely to form a partner preference than males reared in socially enriched environments (BP→GRP). Furthermore, chronic doses of IN-OT over development led to a high degree of alloparental care behavior in the males raised in socially depleted environments (SM→ISO). This result suggests an additive or synergistic effect of a two-hit social deprivation treatment during postnatal development and IN-OT treatment on prairie vole pro-social behavior.

The main effects of social environment on body mass and behavior

Our findings indicate that, regardless of intranasal treatment, the combination of single-mother rearing during perinatal stages and isolated housing during pre-adult stages produced male prairie voles that (i) engaged in more social contact in pre-adolescence (Fig 4.2), and (ii) developed stronger partner preferences in adulthood (Fig 4.4). These SM→ISO males also developed smaller body masses when compared to BP→GRP males, however body mass did not significantly correlate with performance in behavioral tests (Supplementary Table S4.1, Supplementary Figure S4.1a-g). Several reports have separately addressed the effects of perinatal and pre-adult social environments in prairie voles. Single-mother rearing has previously been shown to result in lower body mass at weaning (Wang & Novak, 1992) which is a week earlier than indicated by our results. Ahern and Young (Ahern & Young, 2009) demonstrated that

single-mother reared males did not form partner preferences after one day of cohabitation, instead requiring a week of cohabitation before a preference was formed. This study also found no effect of rearing condition on alloparental care behavior in males. Our results complement this study's reported effect of single-mother rearing on alloparental care (when we exclude SM→ISO+OT; Fig 4.3). Surprisingly, our findings in the Partner Preference test were inconsistent with Ahern & Young (2009), showing that single-mother reared males were more likely to form a partner preference than bi-parentally reared males.

We suspect that the difference in results between our study and Ahern & Young (2009) could be attributable to the impact of social isolation that our design imposed. Other studies have found that pre-adult social isolation leads to an increase in social interaction (Gilles & Polston, 2017; Pan et al., 2009; Wongwitdecha & Marsden, 1996). In rats, this increase in social interaction can be due in part to increases in aggression (Wongwitdecha & Marsden, 1996). Aggression between males in pre-pubertal and sexually naïve prairie voles is rare, which may be why this effect of social isolation was not observed in our study and others. Male prairie voles that experience postweaning group housing, but not those that experience social isolation, show a preference for spending time in an empty cage rather than a cage containing a tethered novel male (Pan et al., 2009). This could reflect an effect of isolated housing on the approach of social novelty (or reduction of social neophobia), as suggested by the increased social contact with a juvenile we observed at PND 35 and PND 42. Pan et al. (Pan et al., 2009) found that isolated male voles exhibited more anxiety in an elevated plus maze, and Grippo and colleagues demonstrated that isolated male voles develop anhedonia when testing for sucrose preference and helplessness in a forced swim test (Grippo, Cushing, & Carter, 2007; Grippo, Gerena, et al., 2007). In the latter

studies, the experience of social isolation occurred during adulthood, after subjects were group housed with siblings during pre-adult development.

Prounis et al. (2015) conducted the only other study to explore the effects of being reared by a single-mother and pre-adult social isolation in sequence. In this study, SM→ISO reared males had greater expression of OTR in specific forebrain regions (see below) and impaired social cognition when compared to BP→GRP males and other males experiencing other combinations of pre-weaning and post-weaning social environment (i.e., SM→GRP and BP→ISO; (Prounis et al., 2015)). Taken together, the effects of single-mother rearing and social isolation, in sequence, produce a social phenotype that is distinct from that found in voles experiencing relatively more social enrichment during perinatal and pre-adult development. This social phenotype may emerge due to developmental differences in OT (see below). We wonder if social deprivation during both perinatal and pre-adult stages drives SM→ISO males to seek positive social interaction, which could serve as a social buffer, when opportunities for positive social interaction are available. If true, this hypothesis would explain our observation of increased social contact by SM→ISO males with juveniles. Such social buffer seeking could facilitate prosocial behaviors that result in an increased likelihood of pair bond formation, similar to the results we found. On the other hand, this idea is inconsistent with the social discrimination test results described by Prounis et al. (Prounis et al., 2015), in which SM→ISO males did not show higher investigation of stimulus animals in a radial arm maze compared to other subject groups.

The relative lack of partner preference in BP→GRP voles was unexpected, as voles reared in these standard conditions typically exhibit partner preference in other studies. It is important to

acknowledge that approximately half of our BP→GRP subjects still exhibited a partner preference (Supplementary Table S4.3a). Indeed, 'typical' partner preference behavior of the prairie vole has been shown to be highly variable (Vogel, Patisaul, Arambula, Tiezzi, & McGraw, 2018). To this point, each experiment of this type should be appreciated as an exploration of differences within the subpopulation of voles used in any individual study.

Synergistic effects of social environment and IN-OT on alloparental care behavior

The unique degree of alloparental care behavior in the SM→ISO+OT males suggests synergistic effect of social environment and exogenous OT (Fig 4.3). In every trial, SM→ISO+OT males exhibited care towards the pups. In contrast, approximately half of the control males (BP→GRP+S) attacked the pups during the trial (Supplementary Table S4.2a-b). Our data show that IN-OT reduces the likelihood of attack behavior during the alloparental test (Supplementary Table S4.2c-d). However, only the SM→ISO+OT males expressed distinctly elevated levels of huddling behavior in the test, and this effect persisted approximately two weeks after the period of intranasal treatment (Fig 4.3). This may be due to a unique effect of social context and exogenous OT on the expression of OT neuron density or OTR.

We previously showed that the same experimental social condition used here (SM→ISO) produces distinct increases of OTR in the lateral septum (LS), prefrontal cortex (PFC), septohippocampal nucleus (SHi), and basolateral amygdala (BLA) when compared to control subjects (BP→GRP) (Prounis et al., 2015). It is possible that this pattern of OTR expression establishes a unique response to our chronic IN-OT application. The lateral septum plays a critical role in maternal behavior. For example, a strain of female knockout mice (B6 *Peg3*-KO)

had lower OTR in the LS and medial preoptic area, exhibited more maternal aggression towards a male intruder, and less maternal care (F. A. Champagne, Curley, Swaney, Hasen, & Keverne, 2009). If our SM→ISO males had more OTR in their LS than BP→GRP (as previously shown in (Prounis et al., 2015)), it is possible that our dose of chronic OT resulted in LS signaling that promoted parental care in these subjects.

Chronic IN-OT may impact mechanisms that prevent an attack response towards pups in SM→ISO males rather than impacting mechanisms that increase huddling behavior. This may be linked to altered processing of social information in the amydala. For example, the central amygdala is specifically implicated in aggression (Bosch & Neumann, 2012). We found that the SM→ISO males have higher OTR density in the basolateral amygdala (Prounis et al., 2015). The BLA is tightly connected and immediately adjacent to the CeA and could aid to suppress attack behavior. In addition, IN-OT in rats doubles extracellular levels of OT in the amygdala and hippocampus (Neumann et al., 2013). This potential combination of increased OT and increased OTR in the amygdala of SM→ISO+OT males provides a plausible mechanism that could facilitate behavioral differences in this group.

It is also pertinent to consider the effects of isolated housing on stress reactivity in male prairie voles. Specifically, after experiencing a resident-intruder test, isolated male prairie voles have higher circulating concentrations of OT in their plasma, and higher activity of OT-reactive neurons in the paraventricular nucleus in response to this social stress (Grippo, Gerena, et al., 2007). This heightened OT response to stress may have been a factor in the present study, where voles were handled daily for three weeks for intranasal application of saline or OT. If this is the

case, the SM→ISO+OT may have experienced a distinctly high amount of OT exposure due to a combination of the heightened stress reactivity and the effects of the IN-OT itself. This interaction of effects on circulating OT and OTR expression may mediate the unique social behavior of the SM→ISO+OT males, which may be interpreted as either increased motivation to interact, or reduced social neophobia. To this point, the effects of post-weaning social isolation on depressive-like behavior can be partly eliminated by intraperitoneal injections of OT (Grippo et al., 2009).

Our study did not address the precise mechanism of action promoting alloparental care behavior in SM→ISO+OT males, but previous research in prairie voles highlights some potential places to explore. OTR in the NAcc is particularly high in juvenile prairie voles, is correlated with alloparental care behavior (Olabazel and Young 2007a) and mediates alloparental care (Olazabal & Young, 2006). Notably, chronic IN-OT has previously been reported to increase OTR in NAcc in females, but not male prairie voles (Guoyones et al. 2018).

Chronic IN-OT has previously been shown to have no effect on alloparental care behavior or juvenile affiliation in standard reared male prairie voles (i.e., BP→GRP) (Bales et al., 2013). In the same study, however, standard reared voles receiving the same dose of IN-OT as we used (and a lower dose) did not form partner preferences; this was not the case for a larger dose than we used (Bales et al., 2013). In large part, our results complement this earlier study, as our BP→GRP+S and BP→GRP+OT males also did not differ in juvenile affiliation or alloparental care behavior. However, we failed to replicate the effect of the medium dose on partner preference behavior. Earlier studies demonstrate the complex relationship between exogenous

OT and behavior, showing highly variable outcomes of alloparental care behavior and partner preference behavior according to four different doses of OT given intraperitoneally shortly after birth in female prairie voles (Bales et al., 2007). This should be taken into consideration, along with the highly variable social behavior expressed in unmanipulated prairie voles reared in standard conditions (Vogel et al., 2018).

Concluding remarks

The effects of social environment on behavioral development, as mediated by changes to nonapeptide systems, is a dynamic area of study with many moving parts. We altered both the social environment and the OT system in developing prairie voles, and in doing so we identified that responses to intranasally administered oxytocin can be mediated by the degree of social deprivation experienced in perinatal and pre-adult development. Our results have translational application to exploring clinical use of IN-OT in children, adolescent, and adult humans. The effects of IN-OT on human behavior are remarkably inconsistent (Bartz, Zaki, Bolger, & Ochsner, 2011; Keech, Crowe, & Hocking, 2018). This generates controversy ranging from people who fear negative long-term effects of chronic IN-OT application, to people who doubt there are any effects at all (Leng & Ludwig, 2016; Young, 2013). We offer a potential source of variable responses to IN-OT, emphasizing consideration for the shaping force of social environment on individual variation of OT.

Future studies must continue to explore how early life social environments across life stages may interactively shape phenotypes. As this foundation of knowledge grows we can begin to form predictions about how social environments can adaptively tune physiological and neural systems.

For example, does a sequence of perinatal social deprivation and juvenile social deprivation tune phenotypes in a way that increases the likelihood of reproductive success in future environments of low population density? Future research should test predictions of adaptive tuning by combining early life manipulations in the lab with fitness outcomes in semi-natural environments. This may demonstrate that, in the proper context, phenotypic outcomes of deprivation otherwise defined as pathological can have adaptive consequences.

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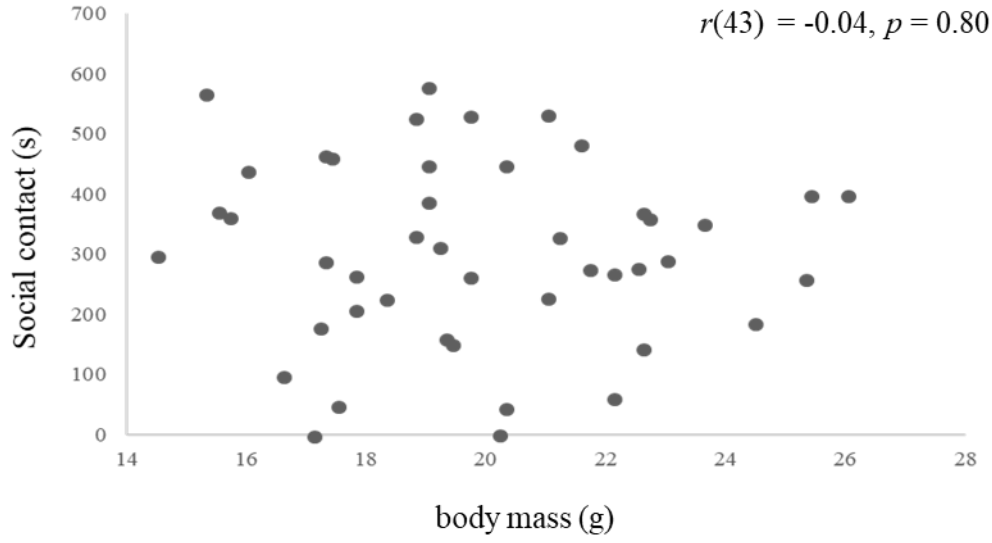
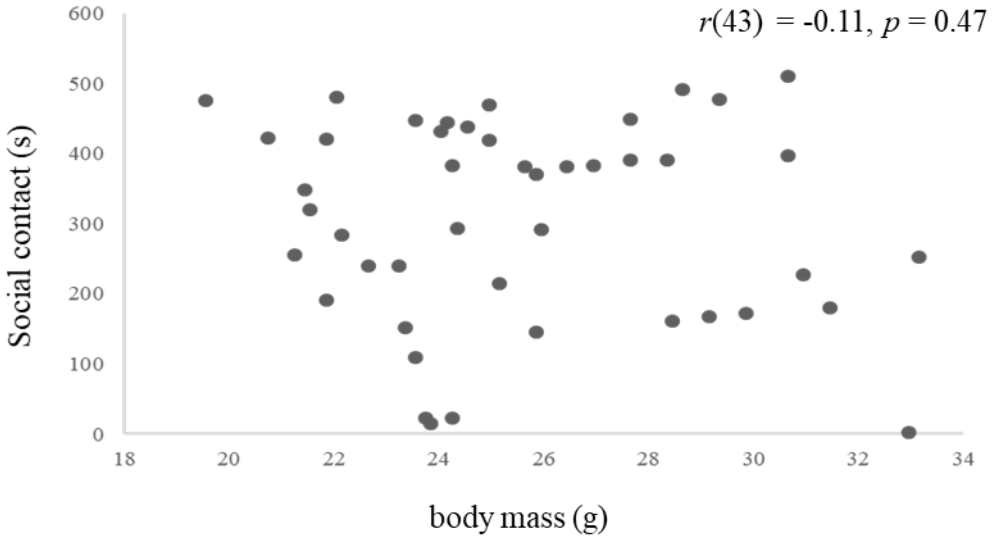
SUPPLEMENTAL MATERIAL

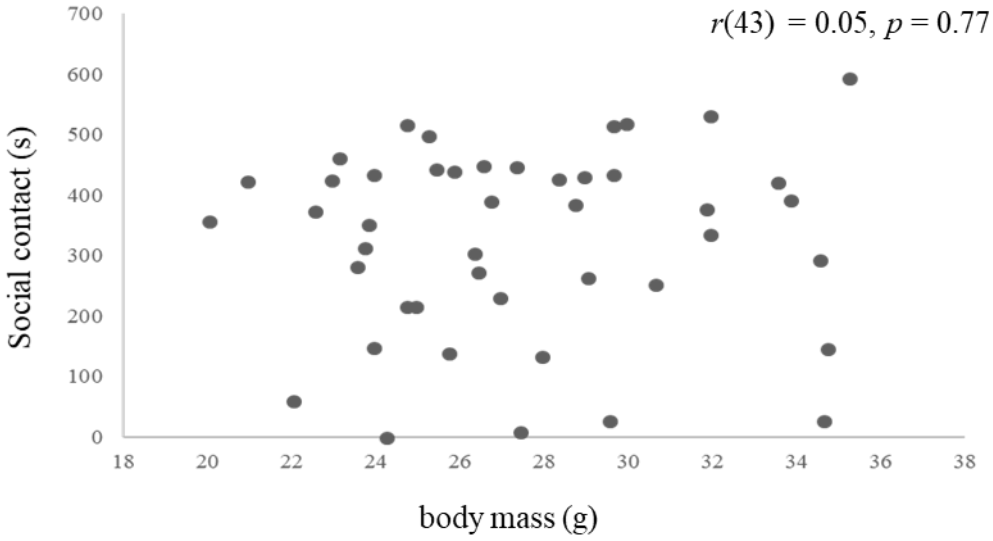
Supplemental Figure S4.1a. Correlation between body mass at PND 21 and social contact time with juvenile in Juvenile Affiliation Test at PND 22.



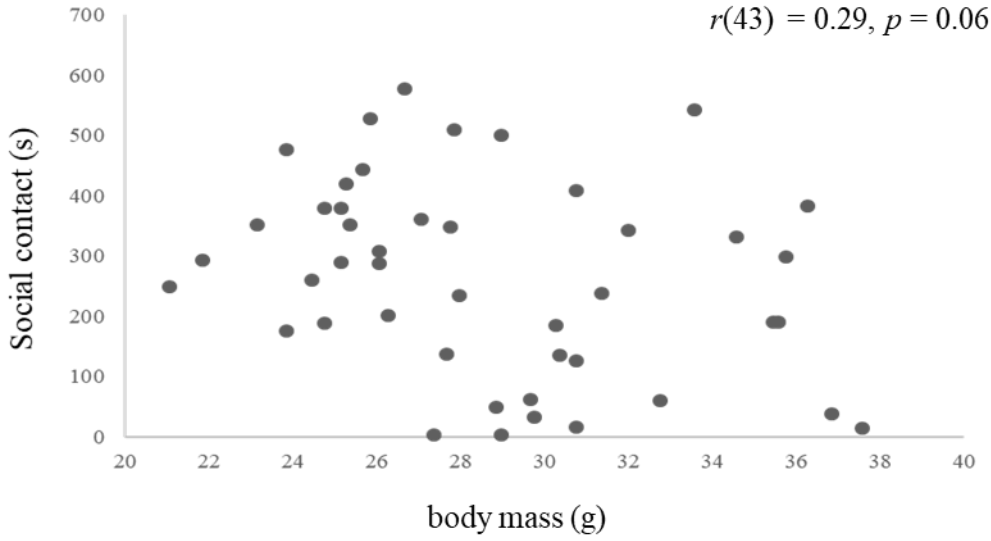
Supplemental Figure S4.1b. Correlation between body mass at PND 28 and social contact time with juvenile in Juvenile Affiliation Test at PND 28.



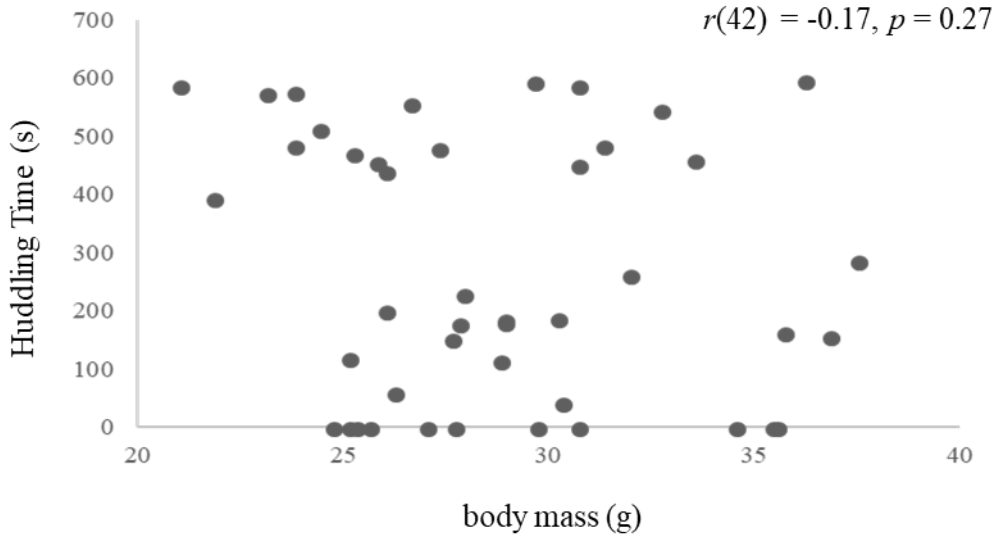
Supplemental Figure S4.1c. Correlation between body mass at PND 35 and social contact time with juvenile in Juvenile Affiliation Test at PND 35.



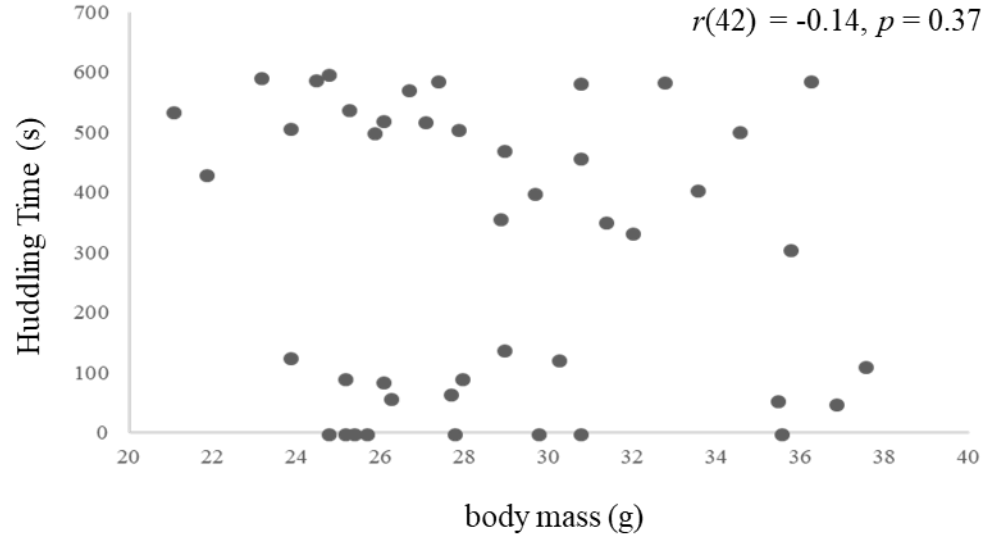
Supplemental Figure S4.1d. Correlation between body mass at PND 42 and social contact time with juvenile in Juvenile Affiliation Test at PND 42.



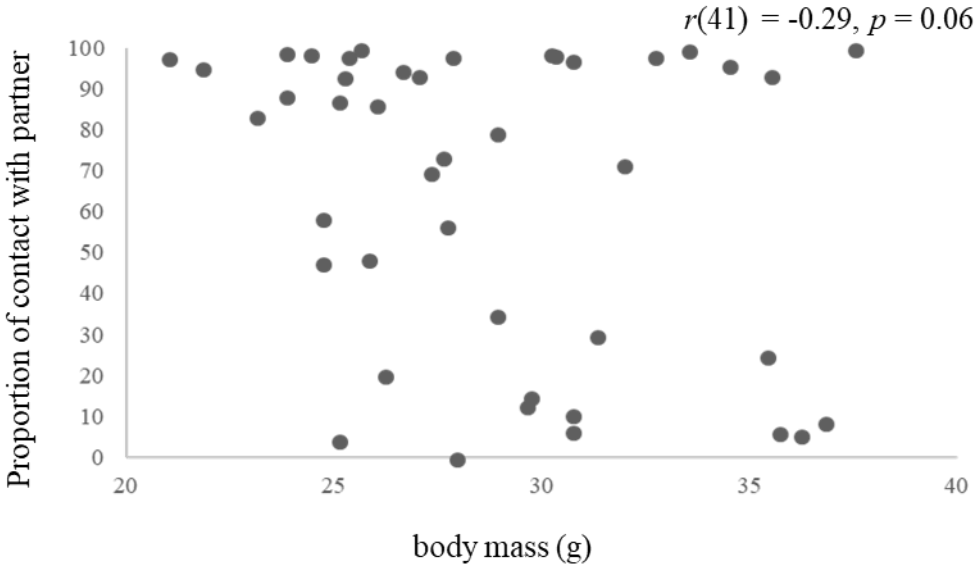
Supplemental Figure S4.1e. Correlation between body mass at PND 42 and huddling in the PND 43 Spontaneous Alloparental Care Test



Supplemental Figure S4.1f. Correlation between body mass at PND 42 and huddling in the PND 58 Spontaneous Alloparental Care Test



Supplemental Figure S4.1g. Correlation between body mass at PND 42 and proportion of time in side-by-side contact with partner in the Partner Preference Test.



Supplemental Table S4.1: Body mass at different stages of post-weaning development according to social manipulation (BP→GRP = biparental→group; SM→ISO = single-mother→isolation) + intranasal treatment group (+S = saline; +OT = oxytocin).

Body Mass (g):		**PND 21 (±SD)**	**PND 28* (±SD)**	**PND 35* (±SD)**	**PND 42* (±SD)**
Group	BP→GRP+S	20.99 (± 2.91)	27.25 (± 3.48)	29.30 (± 3.69)	30.52 (± 4.09)
	BP→GRP+OT	19.54 (± 2.17)	26.02 (± 3.34)	28.12 (± 4.20)	29.59 (± 4.25)
	SM→ISO+S	19.58 (± 3.20)	24.51 (± 3.19)	25.96 (± 3.56)	27.41 (± 3.92)
	SM→ISO+OT	19.29 (± 3.04)	24.70 (± 3.41)	26.43 (± 4.06)	27.38 (± 4.28)

*ANOVA: Main effect of social manipulation, p <0.05

Supplemental Table S4.2a: Association between social manipulation + intranasal treatment and attack behavior in the spontaneous alloparental care test at postnatal day 43. A comparison of all groups.

Variable		Attack(%)	No Attack(%)	n	χ^2-statistic[a](df)	P-value[a]
Group	BP→GRP+S	5 (45.5)	6 (54.5)	11	6.55 (3)	0.09
	BP→GRP+OT	2 (20.0)	8 (80.0)	10		
	SM→ISO+S	3 (25.0)	9 (75.0)	12		
	SM→ISO+OT	0 (0)	11 (100.0)	11		

[a] Chi-square test for independence

Supplemental Table S4.2b: Association between social manipulation + intranasal treatment and attack behavior in the spontaneous alloparental care test at postnatal day 58. A comparison of all groups.

Variable		Attack(%)	No Attack(%)	n	χ^2-statistic[a](df)	P-value[a]
Group	BP→GRP+S	4 (36.4)	7 (63.6)	11	5.87 (3)	0.12
	BP→GRP+OT	1 (11.1)	8 (88.9)	9		
	SM→ISO+S	2 (16.7)	10 (83.3)	12		
	SM→ISO+OT	0 (0)	12 (100.0)	12		

[a] Chi-square test for independence

Supplemental Table S4.2c: Association between intranasal treatment and attack behavior in the spontaneous alloparental care test at postnatal day 43.

Variable		Attack(%)	No Attack(%)	n	χ^2-statistic[a](df)	P-value[a]
Group	OT	2 (9.5)	19 (90.5)	21	3.99 (1)	0.05
	Saline	8 (34.8)	15 (65.2)	23		

[a] Chi-square test for independence

Supplemental Table S4.2d: Association between intranasal treatment and attack behavior in the spontaneous alloparental care test at postnatal day 58.

Variable		Attack(%)	No Attack(%)	n	χ^2-statistic[a](df)	P-value[a]
Group	OT	1 (4.8)	20 (95.2)	21	3.73 (1)	0.05
	Saline	6 (26.1)	17 (73.9)	23		

[a] Chi-square test for independence

Supplemental Table S4.3a: Association between social manipulation and partner preference in the partner preference test.

Variable		Partner Preference (%)	No Partner Preference (%)	n	χ^2-statistic[a](df)	P-value[a]
Group	BP→GRP	9 (45.0)	11 (55.0)	20	3.74 (1)	0.05
	SM→ISO	17 (73.9)	6 (26.1)	23		

[a] Chi-square test for independence

Supplemental Table S4.3b: Association between social manipulation + intranasal treatment and partner preference behavior in the partner preference test. A comparison of all groups.

Variable		Partner Preference (%)	No Partner Preference (%)	n	χ^2-statistic[a](df)	P-value[a]
Group	BP→GRP+S	5 (45.5)	6 (54.5)	11	3.76 (3)	0.29
	BP→GRP+OT	4 (44.4)	5 (55.6)	9		
	SM→ISO+S	8 (72.7)	3 (27.3)	11		
	SM→ISO+OT	9 (75.0)	3 (25.0)	12		
[a] Chi-square test for independence						

Chapter 5 – General Conclusion

Social environments dynamically shape nonapeptide systems over developmental time. This environmentally-induced variation of oxytocin (OT) and vasopressin (VP) phenotypes mediates differences in social behavior between individuals (K. L. Bales & Perkeybile, 2012). My dissertation research has provided novel insight into the interactive effects of social environments at disparate stages of life on the development of OT receptor (OTR), VP 1a receptor (V1aR), and social behavior in the prairie vole (*Microtus ochrogaster*). I expanded on the tradition of exploring the developmental effects of social environments during a single stage of development, and revealed that perinatal (i.e., pre-weaning) and pre-adult (i.e., post-weaning) social environments can interact to uniquely shape neural and behavioral phenotypes.

Summary of major findings

Development of OTR and V1aR expression in the forebrain is region-specific and responsive to the sociospatial environment. In Chapter 2, I provided a thorough description of OTR and V1aR throughout the developing male and female forebrain, revealing region-specific, and occasionally sex-specific, trajectories of receptor development. In regions where receptor densities significantly changed over development, OTR expression increased with age. In contrast, V1aR increased with age, decreased with age, or peaked in expression during juvenile development depending on the region (Fig 2.10). These trajectories were altered in prairie voles living in socially and spatially enriched environments during post-weaning development. In some regions, enrichment was associated with greater OTR density in both males and females. Alternatively, enrichment caused a male-specific reduction of V1aR in several regions (Fig 2.11). Overall, my findings in Chapter 2 suggest that regions across the forebrain are not

following a single model of nonapeptide receptor development. Patterns appear to concomitantly include widespread receptor density reductions and increases as animals age. These region-specific trajectories might provide a mechanism by which specific neural networks become more sensitive than others to OT and VP release during certain stages of development. This may facilitate developmentally-timed expressions of social behavior in an adaptive manner. Further, only some regions have OTR and V1aR densities that flexibly responded to environmental change. This region-specific plasticity to sociospatial complexity suggests adaptively selective responses of specific neural networks to the manipulated factors. It is important to consider that these early age OTR and V1aR densities could represent ontogenetic adaptations (Oppenheim, 1981), rather than strictly reflecting preparatory mechanisms that are building towards adult behaviors and functions. OTR and V1aR phenotypes may adaptively shift over the course of development in response to changing social environments. For example, these phenotypes may facilitate the expression of social behaviors to operate within specific ontogenetic niches, defined by these changing social factors (Alberts, 2008).

Perinatal and juvenile social environments can interactively shape OTR and V1aR expression and impact social cognition. In Chapter 3, I demonstrated that male prairie voles experiencing social deprivation during both perinatal development (i.e., single-mother rearing) and juvenile development (i.e., isolated housing) show higher OTR in the prefrontal cortex, septohippocampal nucleus, and basolateral amygdala (due to a main effect of juvenile environment), and greater V1aR in the retrosplenial cortex and medial amygdala (due to a main effect of perinatal environment) (Fig 3.3). The interaction between perinatal and juvenile environments produced greater OTR density in the lateral septum of these males. These doubly

deprived males also showed impairments in a task that tested for social discrimination when spatial contexts were altered between familiarization and testing phases (Fig 3.2). These results give merit to the importance of considering the synergistic effects of social environments experienced at distinct periods of development. Social environments across the lifetime can alter neural nonapeptide phenotypes in a way that produces a unique behavioral phenotype.

Perinatal and juvenile social environments can interactively shape the behavioral effects of intranasal OT. In Chapter 4, I demonstrated that the effects of social environment on development can influence the response to exogenous OT treatment. After chronic intranasal OT treatment, male prairie voles experiencing the same social deprivation manipulation implemented in Chapter 3 exhibited high levels of alloparental behavior (in tandem with an absence of attack behavior towards pups) (Fig 4.3). These single-mother→isolated (SM→ISO) males also engaged in higher degrees of social contact with juveniles at the later stages of pre-adolescence (Fig 4.2), and exhibited stronger partner preference behavior in adulthood (Fig 4.4). These findings highlight the functional significance of socio-environmentally induced changes to the OT and VP systems in prairie voles. It is plausible that the alterations to OTR and V1aR in SM→ISO males identified in Chapter 3 are responsible for altering the binding rates of intranasal OT within specific neural networks. This could lead to promoting prosocial behavior, or reducing agonistic behavior, in the alloparental care test. The combined effects of environment and intranasal OT may have also altered OTR densities in a way that was not identified previously. This could explain the same observation of prosocial behavior in the second alloparental care test that was implemented in adulthood.

Comparing results between research aims

Major themes have emerged from the results of my dissertation research that call for further interpretation. The developmental trajectories of OTR and V1aR in Chapter 2 provide a foundation for understanding the effects of social environment on these receptors in Chapter 3. For example, OTR density in the prefrontal cortex and the basolateral amygdala increases over the course of development (Fig 2.10) and this seems to be further increased in response to juvenile social isolation (Fig 3.3). In contrast, OTR in the lateral septum and septohippocampal nucleus does not change over development (Fig 2.10), however, being reared by a single-mother and then experiencing social isolation increases OTR in this region (Fig 3.3).

Two qualitatively distinct social manipulations (i.e., deprived versus enriched) resulted in similar or dissimilar changes to OTR or V1aR density, depending on the region in question. The results from Chapter 2 and 3 suggest that some regions would show upregulation of OTR density, whether a vole experienced relative social deprivation or enrichment during post-weaning development. For example, OTR expression in the prefrontal cortex and the basolateral amygdala increased after environmental enrichment (Fig 2.11) and after social deprivation (Fig 3.3). It is difficult to make strong claims about the mechanism behind this result without knowing more about the specific neuronal populations affected in both cases. However, it is interesting that either enriching or depleting social experiences can result in a similar outcome (i.e., upregulation of OTR) within the prefrontal cortex and basolateral amygdala. Both enriching and depleting social experiences have been previously shown to increase OTR binding. For example, female rat pups that receive high amounts of licking and grooming show an increase of OTR binding in the medial preoptic area, bed nucleus of the stria terminalis, paraventricular

nucleus, and amygdala (Champagne & Meaney, 2007). Interestingly, female prairie voles that do not experience typical handling early in life have increased OTR binding in the nucleus accumbens, bed nucleus of the stria terminalis, and lateral septum (K.L. Bales, Boone, Epperson, Hoffman, & Carter, 2011). This trend contrasts with my observation that enrichment reduces V1aR in the medial amygdala (Fig 2.10) whereas single-mother rearing results in promotion of V1aR in this region (Supplemental Fig 3.2). This could suggest that nonapeptide mechanisms in the medial amygdala afford bi-directional response of V1aR density depending on the nature of the social environments that are experienced.

Between Chapter 3 and Chapter 4, I demonstrated a complex effect of social experiences on the social cognition and behavior of SM→ISO males. In Chapter 3, these males did not demonstrate intact social discrimination in a task that tested discrimination after a familiarization phase, between which the familiar animal was rotated to a new location in a radial arm maze. During the testing phase, SM→ISO males did not spend significantly more time investigating the novel animal, which is how this test analyzes social discrimination ability. Instead, this group of males spent more time than other groups in the location where the familiarized animal was previously located. Although we concluded in Chapter 3 that the consequences of social rearing environment on patterns of V1aR and OTR density in the brain accounted for differences in social cognition, the results from Chapter 4 suggest that SM→ISO males were relatively more prosocial. It is possible that differences in prosocial tendencies account for the differences we attributed to cognition deficits in Chapter 3. For instance, SM→ISO males developed stronger partner preferences than controls. Although speculative, it is intriguing to consider that tangential mechanisms operating in Chapter 3 and 4 (e.g., an increase in OTR in the lateral septum) could

be responsible for attachment/preference with the former location of the familiarized male, and attachment to the female partner. Alternatively, the SM→ISO males may show a higher general interest in positive social contact, as was indicated in all three of the social behavior tests implemented in Chapter 4 (note: only males receiving intranasal OT were prosocial in the Alloparental Care test). This increased interest in positive social contact may alter the search pattern of these males in the socio-spatial discrimination task, causing them to spend more time investigating locations that previously afforded social interaction. Reports in other vertebrate taxa have identified a specific connection between OTR in the lateral septum and prosocial behavior. For example, more gregarious species of finches have higher receptors for mesotocin (the avian homolog of OT) in the dorsal lateral septum, and antagonizing these receptors reduces flocking behavior in the zebra finch, a gregarious species (Goodson, Schrock, Klatt, Kabelik, & Kingsbury, 2009). A specific increase of nonapeptide receptor binding in the lateral septum is also found in the field sparrow and dark-eyed junco during the winter when these two species engage in flocking behavior (Wilson, Goodson, & Kingsbury, 2016). The prosocial behaviors of the SM→ISO males in my study may reflect a conserved function of OT-like nonapeptides in the lateral septum, a region where SM→ISO males had an increase in OTR receptor density.

Considerations and directions for future research

Moving forward, it is important to dissect whether the effects of social environments on OTR, V1aR, and social behavior in prairie voles are mediated by the relationship between nonapeptides and monoamine systems, sex steroids, and/or stress mechanisms. The monoamine dopamine, for example, also acts as a neuromodulator in the brain and is associated with attributing reward value to stimuli, including social partners. In the prairie vole, dopamine

receptors in the nucleus accumbens have been causally linked to partner preference formation (Aragona et al., 2006). This is the primary region where manipulations have shown the causal connection between OTR expression and partner preference (Liu & Wang, 2003). During mating, it is believed that OT signaling in the NAcc modifies dopaminergic circuits affecting reward attribution to the partner (Amadei et al., 2017; Johnson & Young, 2017). In Chapter 2, I found that OTR in the NAcc increases over development in both males and females (Fig 2.10). It is plausible that this increase facilitates dopaminergic mechanisms in a way that makes social attachment to non-kin possible. This would suggest that parental and sibling attachments in neonates operate in a unique way that does not require adult levels of OTR in the NAcc.

OT also interacts with serotonin, another monoamine that plays a significant role in regulating social behavior (Cools, Roberts, & Robbins, 2008). For example, intraperitoneal injection of OT results in higher serotonin axon densities in brain regions where V1aR is expressed, including the medial amygdala (Eaton et al., 2012). Indeed, OT can bind to V1aR to regulate behavior (Song et al. 2016). In Chapter 3, I found that V1aR in the medial amygdala is promoted in male prairie voles reared by a single-mother (Supplemental Fig 3.2). The enhancement of prosocial behavior in SM→ISO males, especially those receiving intranasal OT, may be due to effects of OT on serotonin systems, and the effects of this social experience on V1aR in the medial amygdala (Figs. 4.2-4.4). To this point, intranasal OT in macaques has been shown to provoke release of serotonin in limbic areas and increase serotonin 1A receptors (Lefevre et al., 2017).

It is possible that my results are contingent on the relationship between OT and estrogens. For example, early treatment with OT results in long-term changes to estrogen receptor alpha (Kramer, Yoshida, Papademetriou, & Cushing, 2007). Further, OT and estradiol synergistically

produce enhanced anxiolytic effects in the mouse (McCarthy, McDonald, Brooks, & Goldman, 1996). In prairie voles, social buffering after stress is contingent on the effects of OT on corticosterone (Smith & Wang, 2014). As an anxiolytic, the sociobehavioral functions of OT may be partly due to reducing stress responses in novel social situations. This would certainly explain how the potential changes in OTR via environmental manipulation and intranasal OT could lead to the development of more prosocial behaviors in novel situations, as discovered in Chapter 4.

In the future, it would be of immense value to directly explore the adaptive functions of these socioenvironmental effects on behavior and neurobiology. This could involve lab or controlled semi-natural enclosures that expose developing prairie voles to the same conditions implemented in my research. These prairie voles can then be placed into semi-natural enclosures of adjustable ecological parameters to test for the adaptive benefits of certain early experiences within certain environmental contexts in adulthood. Predictions could be made based on concepts such as the 'mismatch hypothesis' (Nederhof & Schmidt, 2012) which states that aversive experiences early in life trigger adaptive processes which may lead to maladaptive phenotypes if the later environment is not accurately predicted by this early adversity For example, I found that male prairie voles that experienced a socially depleted environment during both pre-weaning (i.e., raised by a single mother) and post-weaning (i.e., social isolation) exhibited a region-specific increase in nonapeptide receptors, and a pattern of prosocial behavior and social cognition not observed in groups of animals raised under other contexts. Could the relative matching of these early environments be responsible for the unique phenotype I observed in these males? The success of these males may depend on how well the sociospatial parameters of this early life

condition predict the social environment they will face in adulthood. This premise prompts the question: would SM→ISO males have higher fitness than controls in environments with low density of males to females? If natal environments can reliably provide information about the social environment in which the vole will need to compete as an adult, then adaptive programming of the phenotype is plausible. In this scenario, being raised by a single-mother and having low interaction with siblings could lead to adaptive programming of nonapeptide and behavioral phenotypes to maximize fitness in a low-density population. In Chapter 3, SM→ISO males had a unique combination of OTR and V1aR expression that has previously been found in reproductively unsuccessful males exhibiting a non-monogamous mating tactic ('wandering') in seminatural enclosures (Ophir, Gessel, Zheng, & Phelps, 2012; Ophir, Wolff, & Phelps, 2008). The success of SM→ISO males in a seminatural enclosure may depend on the social parameters within, for example operational sex ratio and population density. Experiments conducted in semi-natural enclosures can be used to test adaptive tuning of SM→ISO males to specific environments, according to the matching between the sociospatial features experienced during both early life and adulthood. Taken together, this framework provides a foundation for making predictions about how early life social environments might predispose animals for fitness advantages in ecologically-relevant contexts.

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